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STUDIES IN THE PHYSIOLOGY OF THE ONION PLANT

III. FURTHER EXPERIMENTS ON THE EFFECTS OF STORAGE TEMPERATURE AND OTHER FACTORS ON ONIONS GROWN FROM SETS

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A wide range of factorial experiments at Wisley on onions grown from sets included combinations of the factors: storage of sets at high and low temperatures during the winter, variety, set size, set planting date and nitrogen manuring. Bolting, yield and ripening data are recorded. One experiment was repeated in Scotland and Northumberland where early ripening was expected to be more important than at Wisley. In general, all the factors enumerated except nitrogen manuring had large effects on bolting and yield, and storage temperature had a large effect on the time of ripening. Varieties can be selected such that large sets may be used without appreciable loss by bolting and maximum yield thus secured. Storage temperatures of from 20 to 24° C., applied for about 14 weeks from October onwards, can be used to control bolting and to increase the yield even of non-bolting strains by delaying ripening and so extending the growth period. Storage at 24° C. is more effective in preventing bolting than the lower temperatures, but 20° C. is probably more effective in delaying ripening. High-temperature storage is probably unsuitable for sets to be planted in the north and west of Great Britain because of the difficulty of ripening the bulbs. For these districts low-bolting strains are therefore essential. Storage at 0° C. gives only a partial control of bolting and has no effect on ripening. Late planting of sets, although very effective in controlling bolting, results in a substantial loss of yield and, therefore, is not a practical measure. Nitrogen manuring had practically no effect on yield and could not be used to prevent the loss of yield caused by late planting.

INTRODUCTION

Thomson & Smith (1938), and Heath (1943*a*, *b*) showed that the 'bolting' (flowering) of onions grown from sets could be controlled, completely or in part, by exposing the sets to special storage temperatures during the dormant period. Blaauw *et al.* (1941, 1944) have published similar findings. Such temperatures had both direct- and after-effects on initiation of inflorescences (Heath & Mathur, 1944). Other experiments (Holdsworth, 1945) showed that the incidence of bolting could be reduced by late planting of the sets, though at a sacrifice of yield, and it seemed possible that heavy nitrogen manuring might remedy the disadvantage while retaining the advantage of late planting. The experiments described in this paper were initiated to enable the best conditions of storage

temperature, set size, planting date and nitrogen manuring to be more closely defined. A number of varieties of onion was included, and in all the experiments factorial designs were used (Yates, 1937) to elucidate the interactions between factors as well as their average effects. The data for yield and bolting have been subjected to analysis of variance but this has not been done for subsidiary data on maturity. The data are very extensive and can only be presented here in summarized form; except where otherwise mentioned, only clearly significant results are discussed.

EXPERIMENTAL DATA

EXPERIMENT I

1941–2 (*Wisley*). *Set size, storage temperature, planting date and nitrogen dressing*

Design and method of analysis

Commercially produced sets of variety Ebenezer of *three sizes* (< 2, 2–4 and 4–7 g.*) were stored for 16 weeks from 12 November to 2 March at each of *three temperatures* (0° C., a 'common' storage temperature of about 7° C. and 24° C.), planted in the field on *three dates* (9 March, 10 April and 6 May) and given *three levels of nitrogen* manuring (0, 302 and 604 lb./acre of ammonium sulphate). There were thus 3^4 or eighty-one combination treatments, which were not replicated but planted out in nine blocks of nine treatments, the three-factor interactions being confounded with blocks (Yates, 1937, p. 47). No attempt has been made to recover information as to these three-factor interactions; only two-factor interactions and main effects are considered. In the analysis of variance for yield, main effects have been compared with significant two-factor interactions in which they are involved, if any (see Heath, 1943*a*, p. 215), and owing to the accidental omission of one plot at planting, a 'missing plot technique' (Allen & Wishart, 1930; Yates, 1933) has been applied. For analysis of percentage bolting data, the angular transformation (Cochran, 1938) has been used. The data presented in § (*c*) (Ripening) (p. 477) have not been subjected to analysis of variance. Each plot consisted of twenty plants.

Technique

The plot used carried in previous years a succession of vegetables, and the soil was light in texture with a low nitrogen content. The sets were lightly pressed into the soil in rows. The sulphate of ammonia was applied in drills, $\frac{1}{2}$ in. deep, along the sides of the rows 2 in. from the sets. The yield, from all treatments, was small.

Results

(*a*) *Bolting*. Only one of the plants grown from 'small' sets (< 2 g.) bolted; the following discussion and mean data refer to the 'medium' (2–4 g.) and 'large' (4–7 g.) size classes only. The average effect of nitrogen manuring on percentage

* This is very roughly equivalent to $< \frac{1}{2}$, $\frac{1}{2}$–$\frac{3}{4}$ and $\frac{3}{4}$–$\frac{7}{8}$ in. diameter.

bolting was very small and non-significant; the average effects of the other three factors studied were large and important (Table 1).

TABLE 1. *Experiment I*, 1941–2 (*Wisley*). *Mean percentage bolting by 2 July* 1942. *Average effects of single factors*

Set size		Storage temperature		Planting date	
Large (4–7 g.)	15·7	0° C.	11·3	9 Mar.	20·5
Medium (2–4 g.)	3·5	7° C.	13·2	10 Apr.	6·5
		24° C.	4·4	6 May	1·9

Significant differences*: Large > medium. 24 < 0 and 7° C. Early planted > medium > late.

* When compared with random variation, but not with interactions.

The effect of set size is further demonstrated by the negligible bolting in the < 2 g. class. High-temperature storage (24° C.) considerably reduced bolting, but the effect of cold storage (0° C.) was small and non-significant. Late planting effectively suppressed flowering.

Of interactions between pairs of factors, that between set size and planting date was large and highly significant (Table 2(*a*)).

TABLE 2. *Experiment I*, 1941–2 (*Wisley*). *Mean percentage bolting by 2 July* 1942. *Two-factor interactions*

(*a*) Interaction of set size and planting date

	Planted on		
Set size	9 Mar.	10 Apr.	6 May
Large (4–7 g.)	34·6	9·5	3·1
Medium (2–4 g.)	6·4	3·5	0·6

(*b*) Interaction of set size and storage temperature

	Storage at		
Set size	0° C.	7° C.	24° C.
Large (4–7 g.)	19·4	21·9	5·9
Medium (2–4 g.)	3·2	4·5	2·8

(*c*) Interaction of storage temperature and planting date

	Storage at		
Planted	0° C.	7° C.	24° C.
9 Mar.	26·8	27·3	7·5
10 Apr.	6·0	8·0	5·4
6 May	0·9	4·3	0·0

The flowering of the large sets, which presumably had more inflorescence initials at planting time, was suppressed by late planting to a much greater extent than that of the medium sets.

The interaction between set size and storage temperature was somewhat similar (Table 2(*b*)). High-temperature storage reduced the bolting of large more than of

medium sets, the former having more potential bolting to be inhibited—here by the *prevention* of initiation (Heath & Mathur, 1944).

Although the interaction between storage temperature and planting date failed to reach significance, the data are presented (Table 2(*c*)) because of their relation to yield. They suggest that late planting had suppressed the bolting of sets stored at 0 and 7° C. more than at 24° C., since most of the latter had already been prevented from bolting by heat treatment.

(*b*) *Yield.* The data are presented as mean yield per plant, including bulbs which bolted but none which were diseased. The significant average effects (set size and storage temperature) are given in Table 3. Large sets gave a 50% greater yield than medium in spite of more bolting (Table 1). Cold storage had no average effect. High-temperature storage doubled yield, partly no doubt by preventing bolting, but mainly by delaying ripening (p. 477) and perhaps bulbing also (Heath, 1943*b*). The very small yields throughout the experiment were at least partly attributable to premature ripening (p. 478).

TABLE 3. *Experiment I*, 1941–2 (*Wisley*). *Mean yield, g./plant. Average effects of single factors*

Set size		Storage temperature	
Large (4–7 g.)	36·0	0° C.	20·2
Medium (2–4 g.)	23·7	7° C.	20·2
Small (<2 g.)	20·3	24° C.	39·6
S.E. of a single mean			=1·7
Significant difference between two means, P 0·05			=4·8

TABLE 4. *Experiment I*, 1941–2 (*Wisley*). *Mean yield, g./plant. Two-factor interactions*

(*a*) Interaction of storage temperature and planting date

	Storage at		
Planted	0° C.	7° C.	24° C.
9 Mar.	25·7	22·6	53·0
10 Apr.	16·4	19·9	36·0
6 May	18·3	17·9	29·9

(*b*) Interaction of nitrogen dressing and planting date

	Nitrogen dressing		
Planted	Nil	1 (302 lb.)	2 (604 lb.)
9 Mar.	29·9	40·5	30·9
10 Apr.	27·1	28·0	17·2
6 May	25·3	18·7	22·1
S.E. of a single mean			=2·9
Significant difference between two means, P 0·05			=8·2

Two of the two-factor interactions were significant and are shown in Table 4. The interaction of storage temperature and planting date (Table 4(*a*)) showed no

significant differences between 0 and 7° C. storage (as in the average effect—Table 3) or between the last two plantings. The effect of high temperature, which increased yield for all planting dates, was, however, greatly enhanced by early planting. Early planting permits emergence of inflorescences from sets stored at 0 and 7° C. and so shows up the inhibition of flowering in those stored at 24° C. (Table 2(*c*)). These results suggest a potentially very large effect of early planting in improving yield, which in the 0 and 7° C. sets is largely counteracted by increased bolting. The increased yield due to heat treatment (24° C.) would then be due to delay of bulbing and ripening in the case of the second and third plantings, but in the first planting to prevention of flowering in addition.

The interaction between nitrogen and planting date (Table 4(*b*)) showed no significant effect of planting date in the absence of a nitrogen dressing, nor of nitrogen with very late planting. For the second planting date, nitrogen (double dressing) had a depressing effect only. With early planting, a single dressing of nitrogen increased yield by a third, but a double dressing was without effect.

(*c*) *Ripening.* Two measures of ripening were obtained: the mean number of green leaves per plant on 2 July 1942, when the leaves of some plants were already dying off, and the percentage of *bulbs* that had some green leaf at harvest (19 August 1942). The first of these might be expected to show up a nitrogen effect in prolonging the life of the leaves, but, in fact, the average nitrogen effect was negligible. Planting date was also without appreciable average effect, but increasing set size caused a small but fairly consistent increase. The average effect of storage temperature was relatively large, the mean numbers being: 0° C., 3·4; 7° C., 3·3; 24° C., 4·9. Storage temperature was also involved in a fairly large interaction with planting date (Table 5). The effect of 24° C. storage in increasing leafiness and delaying

TABLE 5. *Experiment I*, 1941–2 (*Wisley*). *Mean number of green leaves per plant, 2 July* 1942. *Interaction of storage temperature and planting date*

	Storage at		
Planted	0° C.	7° C.	24° C.
9 Mar.	3·1	2·6	5·5
10 Apr.	3·5	3·5	4·8
6 May	3·6	3·7	4·4

ripening was apparently greater with early planting. This interaction thus resembles, and might seem to account for, the similar interaction in terms of yield (Table 4(*a*)). Here again, however, the effects of bolting (Table 2(*c*)), must be taken into account. Bolted plants would tend to have fewer green leaves than others, and an explanation for the effects on green-leaf number similar to that already advanced for yield (see above) would meet the facts.

The percentage of bulbs with some green leaf at harvest provides a more satisfactory measure. Here again, the only consistent and large average effect was that of storage temperature (Table 6). The average nitrogen effect was negligible, and

there was very little interaction between storage temperature and planting date. The consistent effect of 24° C. storage in delaying ripening is apparent, but there is no increase of effect with early planting as in Tables 4(*a*) and 5. The striking interaction in those tables cannot therefore be due to a much greater delay of ripening by 24° C. storage when combined with early planting. The explanations based on the bolting results (Table 2(*c*)) are more probably correct. Of the plants from 0 and 7° C. sets, very few had any green leaf left at harvest, and even of those stored at 24° C. nearly two-thirds were dead ripe, i.e. ripening was premature throughout.

TABLE 6. *Experiment I*, 1941–2 (*Wisley*). *Mean percentage of bulbs with some green leaf at harvest*, 19 *August* 1942. *Interaction of storage temperature and planting date*

	Storage at		
Planted	0° C.	7° C.	24° C.
9 Mar.	8	9	36
10 Apr.	8	6	37
6 May	6	5	37

EXPERIMENT II

1941–2 (*Wisley*). *Variety, set size, storage temperature and nitrogen dressing*

Design and analysis

Small onions picked out from ordinary commercial field crops of the *two varieties*, Bedfordshire Champion and Best of All, were used. They were graded into *three sizes* (2–4, 4–7 and 7–10 g.), and it should be noted that these included a 'very large' class (7–10 g.) but omitted the 'small' class (< 2 g.) of Exp. I. The *three storage treatments* and the *three levels of nitrogen* manuring were as in Exp. I, but all the sets were planted on the 'early' planting date (9 March). There were thus 2×3^3 or fifty-four combination treatments, and these were planted in duplicate giving 108 plots, each of fifteen plants. Within each replication the plots were divided into three blocks of eighteen with the three-factor interaction, size × storage temperature × nitrogen dressing, confounded. Only the main effects and two-factor interactions are discussed. The methods and application of analysis of variance have been similar to those in Exp. I.

Experimental technique and field notes

The technique closely followed that adopted in the preceding experiment except that in this and all subsequent experiments the sets were planted in drills, 1 in. deep.

The growth of the sets was highly satisfactory and the resulting onions were of good size.

Results

(*a*) *Bolting*. The average effect of nitrogen dressing was again very small and entirely non-significant. The other three factors gave average effects of considerable magnitude (see Table 7). The size effect was of the usual nature. The effects of both

high- and low-temperature storage were larger than in Exp. I; this was true even for the same size and planting date classes, in which the bolting from 7° C. sets was about the same in both experiments.

TABLE 7. *Experiment II*, 1941–2 (*Wisley*). *Mean percentage bolting by 2 July* 1942. *Average effects of single factors*

Variety		Set size		Storage temperature	
Bedfordshire Champion	30·8	Very large (7–10 g.)	47·6	0° C.	33·5
Best of All	22·9	Large (4–7 g.)	25·5	7° C.	41·2
		Medium (2–4 g.)	7·4	24° C.	5·8

Significant differences: Bedfordshire Champion > Best of All.* Very large > large > medium. 24 < 0 and 7° C.

* When compared with random variation, not with interactions.

There was a significant interaction between variety and set size (Table 8(*a*)) such that the varietal difference was greater for the very large sets than for the other sizes.

The interaction between size and storage temperature (Table 8(*b*)) was large and highly significant. Cold storage had no effect on the very large sets, but an appreciable effect on the smaller sizes. The effect of high temperature was much greater for the large and very large than for the medium sets, since the latter had less potential bolting to be inhibited (as in Exp. I, p. 475).

TABLE 8. *Experiment II*, 1941–2 (*Wisley*). *Mean percentage bolting by 2 July* 1942. *Two-factor interactions*

(*a*) Interaction of variety and set size

	Very large (7–10 g.)	Large (4–7 g.)	Medium (2–4 g.)
Bedfordshire Champion	54·7	27·0	10·5
Best of All	40·5	24·0	4·2

(*b*) Interaction of set size and storage temperature

	Storage at		
Set size	0° C.	7° C.	24° C.
Very large (7–10 g.)	65·8	64·5	12·7
Large (4–7 g.)	27·0	45·3	4·1
Medium (2–4 g.)	7·6	13·8	0·6

(*b*) *Yield.* The yield data are presented in the same form as for Exp. I (p. 476). The average effects of nitrogen and of variety were entirely non-significant, as were also all the first-order interactions involving either of these two factors. The *average* effect of set size also failed to reach significance; that of storage temperature is shown in Table 9. High-temperature storage nearly trebled the yield, again presumably by its effects in delaying ripening (p. 480) and probably bulbing also (Heath, 1943*b*), and by preventing bolting (Table 7). Cold storage was virtually without effect.

TABLE 9. *Experiment II*, 1941–2 (*Wisley*). *Mean yield, g./plant. Average effects of single factors*

Storage temperature	
0° C.	41·8
7° C.	38·7
24° C.	109·4

S.E. of a single mean = 4·2
Significant difference between two means, P 0·05 = 16·4

The interaction of size and storage temperature is shown in Table 10. The effect of high temperature was less for the medium than for the larger sets, presumably because of the smaller amount of potential bolting to be inhibited (Table 8(*b*)). With bolting controlled by high temperature the effect of large set size in increasing yield became operative, thus giving a significant difference between medium and larger sets. Exp. I showed no interaction of this type affecting yield, even if the later plantings and small-set class were omitted, but in that experiment large sets gave a greatly increased yield in spite of increased bolting and hence there was a significant *average* effect of size (Table 3).

TABLE 10. *Experiment II*, 1941–2 (*Wisley*). *Mean yield, g./plant. Two-factor interactions. Interaction of set size and storage temperature*

	Storage at		
Set size	0° C.	7° C.	24° C.
Very large (7–10 g.)	44·8	40·7	117·4
Large (4–7 g.)	42·9	38·8	118·7
Medium (2–4 g.)	37·8	36·7	92·2

S.E. of a single mean = 4·3
Significant difference between two means, P 0·05 = 12·1

(*c*) *Ripening*. The percentages of bulbs (including bolters) with some green leaf remaining at harvest showed no consistent effects of variety, or nitrogen manuring. There was a small but consistent average effect of size (medium, 26·2%; large, 21·8%; very large sets, 18·2%) which agrees with the findings of Holdsworth (1945) and is similar to the difference between sets and seedlings (Heath, 1943*b*), and a very large average effect of storage temperature. The effect of 24° C. storage was greatest for the medium sets and least for the very large sets, giving an interaction which is given in Table 11. This interaction is more or less the converse of those in

TABLE 11. *Experiment II*, 1941–2 (*Wisley*). *Mean percentage of bulbs with some green leaf at harvest*, 19 *August* 1942. *Interaction of set size and storage temperature*

	Storage at		
Set size	0° C.	7° C.	24° C.
Very large (7–10 g.)	2	4	55
Large (4–7 g.)	6	5	65
Medium (2–4 g.)	11	11	79

terms of yield (Table 10) and bolting (Table 8(*b*)), and hence the interaction effects of these two factors on yield should be attributed to their effects on bolting rather than on delay of ripening. The enormously greater *average* yield following high-temperature storage (Table 9) is doubtless attributable to the combined effects of reduced bolting *and* delayed ripening.

As compared with Exp. I, premature ripening for the same size and planting date classes was almost exactly the same with 0 and 7° C. stored sets, but only half as prevalent for those stored at 24° C. (Tables 6 and 11). This showed up in the yields, which were almost the same in both experiments for large sets stored at 0 and 7° C., but nearly twice as large in Exp. II for sets stored at 24° C. (Table 12).

TABLE 12. *Experiments I and II*, 1941–2. *Mean yields, g./plant, from medium and large sets planted* 9 *March* 1941 *after storage at different temperatures*

		Storage at		
	Set size	0° C.	7° C.	24° C.
Exp. I:	Large (4–7 g.)	41·4	35·6	66·9
	Medium (2–4 g.)	23·4	18·0	48·8
Exp. II:	Large (4–7 g.)	42·9	38·8	118·7
	Medium (2–4 g.)	37·8	36·7	92·2

EXPERIMENT III

1941–2 (*Wisley*). *Variety, set size and storage temperature*

Design and analysis

In all, *seven varieties* were included in this experiment: Ebenezer, Australian Brown, Southport Red Globe, Bedfordshire Champion, Best of All, Red Wethersfield and Southport Yellow Globe (varieties 1–7 respectively), of which 1, 3, 6 and 7 were raised at Wisley from American seed, the rest being small onions picked out from commercial crops in the Home Counties. Varieties 6 and 7, being represented by fewer sets, were graded only into 'medium' and 'large', the others making *three size classes*: 'small' (< 2 g.), 'medium' (2–4 g.) and 'large' (4–7 g.). The *three storage-temperature* treatments were again 0, 7 and 24° C. There was sufficient material for duplicates of the treatments in varieties 1 and 5, so that the total number of plots (of twenty plants) was seventy-five ($3 \times 3 \times 7$ plus $2 \times 3 \times 2$), of which a single randomized block was made. A 'missing plot' technique had, however, to be applied to the treatment 'Variety 3 × medium size × 24° C. storage', as insufficient sets remained after severe storage losses.

Experimental technique and field notes

The method was similar to that in the two preceding experiments. The sets were removed from the controlled temperatures on 3 March and planted on 10 March. Growth of the sets was satisfactory. The crops were harvested in August and weighed on 20 August.

Results

(*a*) *Bolting.* The data for 'large' and 'medium' size classes only have been analysed, as bolting was negligible among plants from small sets. The average effect of variety on bolting was very highly significant (Table 13), but of size and storage

TABLE 13. *Experiment III*, 1941–2 (*Wisley*). *Mean percentage bolting by 3 July 1942. Average effects of single factors*

Variety		
7	Southport Yellow Globe	46·1
3	Southport Red Globe	42·2
6	Red Wethersfield	40·8
2	Australian Brown	26·0
4	Bedfordshire Champion	18·8
1	Ebenezer	11·8
5	Best of All	10·1

Significant differences: 7, 3 and 6 > 2 and 4 > 1 and 5

Set size		Storage at	
Large (4–7 g.)	22·1	0° C.	29·2
Medium (2–4 g.)	7·1	7° C.	35·6
		24° C.	7·9

Significant differences*: Large > medium. 24 < 7 > 0° C.

* When compared with random variation, but not with interactions.

temperature only when compared with the random variance. The interaction size × storage temperature produced a highly significant effect (Table 14). The effect of cold storage just failed to reach significance (P 0·05) even in the medium-size class, where the depression of bolting compared with common storage was the greater; but the effect of high-temperature storage was marked and of the usual kind, the difference being much greater in the large-size class consistent with the greater potential bolting in this class.

TABLE 14. *Experiment III*, 1941–2 (*Wisley*). *Mean percentage bolting by 3 July 1942. Two-factor interaction—set size and storage temperature*

	Storage at		
Set size	0° C.	7° C.	24° C.
Large (4–7 g.)	45·9	51·1	10·1
Medium (2–4 g.)	12·6	19·9	5·7

(*b*) *Yield.* The average effect of variety was not significant even when tested against the random variation. The effects of set size and storage temperature were large, the latter at $P = 0{\cdot}001$ (Table 15). The effects of high and low storage temperature agree very closely with those of Exp. II, in which the yields, generally, were of the same magnitude. The average effect of cold storage was again negligible.

The only significant interaction was that between variety and storage temperature in which only the effect of high-temperature storage was of importance. The largest

TABLE 15. *Experiment III*, 1941–2 (*Wisley*). *Mean yield, g./plant. Average effects of single factors*

Set size		Storage at	
Large (4–7 g.)	61·3	0° C.	40·7
Medium (2–4 g.)	54·7	7° C.	37·2
		24° C.	96·2
S.E. of a single mean	=2·7	S.E. of a single mean	= 3·3
Significant difference between two means, *P* 0·05	=8·0	Significant difference between two means, *P* 0·05	=22·1

yields after treatment at 24° C. tend to be produced by the varieties giving the smallest yields when the sets were stored at ordinary temperatures (Table 16; note, however, that the high yield of variety 3 is the 'missing plot' value and, therefore, doubtful). The varieties 3 and 7 are those which bolted most after storage at 7° C., and it is, therefore, likely in these that the effect of high-temperature storage was mainly a result of its effect on bolting. As shown below, however, there was also a contributory effect on earliness, and this was presumably predominant with varieties 4 and 5 which gave relatively little bolting after ordinary storage. High-temperature storage had less effect on the bolting of variety 6 than on that of any other, and this may explain the low yield of this bad bolting strain even after high-temperature treatment.

TABLE 16. *Experiment III*, 1941–2 (*Wisley*). *Mean yield, g./plant. Two-factor interaction—variety and storage temperature*

	Storage at		
Variety	0° C.	7° C.	24° C.
1	48·1	51·4	75·4
2	41·4	45·3	58·9
3	22·6	15·2	115·9
4	43·4	36·3	95·6
5	55·7	32·2	140·2
6	33·9	26·1	66·5
7	21·7	28·2	149·1
S.E. of a single mean			= 9·8
Significant difference between two means, *P* 0·05			=29·4

(*c*) *Ripening*. The percentage of bulbs with green leaf at harvest (20 August 1942) showed the usual large effect of high-temperature storage in delaying ripening but no effect of low temperature was evident. The means were: 9 and 10% bulbs with green leaf after storage at 0 and 7° C. compared with 52% after 24° C. treatment. All the varieties showed the effect clearly, but the differences due to storage temperature were small in the late varieties because, for these, the count was made too early for much ripening even after storage at ordinary temperatures. The effect of set size is shown in the means of Table 17, but it was not consistent among the varieties. Table 17 also suggests that Southport Yellow Globe, Southport Red

Globe and Red Wethersfield are early varieties, but as these show most bolting, and leaf production is disturbed by the formation of the flower-stalk, their apparent earliness is probably due to the smallness of the number of leaves produced by the bolted plants.

TABLE 17. *Experiment III*, 1941–2 (*Wisley*). *Mean percentage of bulbs with green leaf at harvest*, 20 *August* 1942. *Average effects of single factors*

Variety		
4	Bedfordshire Champion	29
5	Best of All	25
2	Australian Brown	20
1	Ebenezer	19
6	Red Wethersfield	13
3	Southport Red Globe	12
7	Southport Yellow Globe	7
	Set size	
	Large (4–7 g.)	24
	Medium (2–4 g.)	18
	Small (< 2 g.)	30

EXPERIMENT IV

1942–3 (*Wisley, Cockle Park and Auchincruive*). *Variety, storage temperature (including duration of treatment) and set size*

Design and analysis

This experiment was carried out at *three centres*: Wisley, Cockle Park (Northumberland) and Auchincruive (west of Scotland) to compare the growth of sets in the colder and wetter areas of the country where early ripening is likely to be of primary importance. Sets of *two varieties*, Ebenezer and Red Wethersfield, raised at Wisley, were graded into *three size classes*: large (4–7 g.), medium (2–4 g.) and small (< 2 g.). The storage-temperature treatments formed a 3×3 factorial arrangement, being considered as *three levels of high-temperature* treatment (18, 21 and 24° C.) *given for* 0, 7 *or* 14 *weeks* from the beginning of the storage period (23 November 1942); the rest of the 14 weeks' storage was at common temperatures (about 7° C.). Thus the control treatment, i.e. common storage temperature throughout, occurred three times as often as any other treatment. The number of treatment combinations at each centre was 2×3^3, that is, fifty-four. At Wisley all the treatments were duplicated; for each of the other centres data are presented for a single replicate only. Each replicate was divided into three blocks so as to confound the three-factor interaction 'size × temperature × duration of temperature' with blocks. As in previous experiments, only single and two-factor effect have been tested for significance. There were fifteen plants to a plot.

Experimental technique and field notes

Methods were essentially the same as in previous experiments. At Wisley planting was completed on 11 March, at Auchincruive on 6 April and at Cockle Park on 8 April. At Wisley growth was more satisfactory than in 1942 and a good

crop was obtained. At Auchincruive and Cockle Park the crops were even heavier, but many of the plants remained leafy all through the summer and had eventually to be harvested green.

Results

(*a*) *Bolting*. The amount of bolting in Ebenezer was negligible at all three centres. The average percentage bolting for the large-size class in the control treatments was only 3% at Wisley, 19% at Cockle Park and 24% at Auchincruive. Only the Red Wethersfield data, therefore, have been subjected to statistical analysis. At each centre the usual set-size effect was clearly demonstrated (Table 18). The only other

TABLE 18. *Experiment IV*, 1942–3. *Mean percentage bolting by 24 June 1943 (28 June 1943 at Cockle Park). Average effects of single factors. Variety Red Wethersfield*

(*a*) Set size

	Large (4–7 g.)	Medium (2–4 g.)	Small (< 2 g.)
Wisley	60·8	31·4	4·3
	Significant differences: Large > medium > small		
Cockle Park	78·2	42·0	1·4
	Significant differences: Large > medium > small		
Auchincruive	72·5	39·6	6·8
	Significant differences: Large > medium > small		

main effect found significant when tested against the appropriate interaction was duration of storage temperature, and this only at Auchincruive. When tested against the random variance, however (Table 19), this treatment gave a significant effect at

TABLE 19. *Experiment IV*, 1942–3. *Mean percentage bolting by 24 June 1943 (28 June 1943 at Cockle Park). Average effects of single factors. Variety Red Wethersfield*

(*b*) Duration of high-temperature storage

	Storage for (weeks)		
	0	7	14
Wisley	32·1	34·0	20·1
	Significant differences*: 0 and 7 > 14 weeks		
Cockle Park	41·2	41·3	24·6
	Significant difference: None		
Auchincruive	46·8	43·4	22·1
	Significant differences: 0 and 7 > 14 weeks		

* When compared with random variation, but not with interactions.

Wisley also. The average effect of level of high-temperature storage was everywhere very small (Table 20), but this is hardly surprising in view of the design of the experiments. By making a factorial of the temperatures and their durations each main temperature effect is diluted by the inclusion of one-third of control plots.

TABLE 20. *Experiment IV*, 1942–3. *Mean percentage bolting by 24 June 1943 (28 June 1943 at Cockle Park). Average effects of single factors. Variety Red Wethersfield*

(*c*) Level of high-temperature storage

	Storage at 18° C.	Storage at 21° C.	Storage at 24° C.
Wisley	33·0	30·8	22·4
	Significant difference*: 18 > 24° C.		
Cockle Park	28·4	42·9	35·3
	Significant difference: None		
Auchincruive	36·8	42·0	32·4
	Significant difference: None		

* When compared with random variation, but not with interactions.

The effects of storage temperature may, therefore, be expected to show more clearly in the interaction level × duration of high-temperature treatment (Table 21)

TABLE 21. *Experiment IV*, 1942–3. *Mean percentage bolting by 24 June 1943 (Cockle Park, 28 June 1943). Two-factor interaction—level and duration of high-temperature treatment. Variety Red Wethersfield*

Storage at	Weeks' treatment 0	7	14
	Wisley		
18° C.	32·0	34·5	32·8
21° C.	25·6	37·0	30·0
24° C.	39·3	30·5	4·8
	Cockle Park (no statistically significant effect)		
18° C.	29·8	28·2	27·5
21° C.	47·0	53·6	28·5
24° C.	46·8	42·6	18·2
	Auchincruive (no statistically significant effect)		
18° C.	42·8	32·9	35·0
21° C.	51·0	53·4	22·9
24° C.	46·6	44·4	11·0

which was significant at Wisley. In all cases it is seen that 7 weeks' treatment is practically valueless in reducing bolting, and that of the different high temperatures only 24° C. had a noticeable effect. There is some indication, however, that a slight reduction of bolting was caused by 14 weeks' treatment at 21° C. at Auchincruive. The reduction of bolting by 24° C. at the northern centres was less than at Wisley.

None of the other two-factor interactions produced significant effects.

(*b*) *Yield.* The highest yields were obtained in Scotland, the average yields not differing greatly between Cockle Park and Wisley. The main effects on yield are shown in Table 22. Ebenezer gave a bigger yield at all centres than Red Wethersfield, although the difference was not a significant one at Wisley. Except in Scotland,

TABLE 22. *Experiment IV*, 1942–3. *Mean yield, g./plant. Average effects of single factors*

	Wisley	Cockle Park	Auchincruive
(*a*) Variety			
Ebenezer	142·5	166·7	255·2
Red Wethersfield	111·2	96·7	150·3
S.E. of single mean	3·5	5·4	6·6
Significant difference, *P* 0·05	None	82·5	94·6
(*b*) Set size			
Large (4–7 g.)	151·1	147·1	205·5
Medium (2–4 g.)	130·1	135·8	203·1
Small (< 2 g.)	99·5	112·5	199·6
S.E. of single mean	4·3	6·6	8·1
Significant difference, *P* 0·05	31·3	20·1	None
(*c*) Duration of high-temperature storage			
None	119·7	105·7	172·4
7 weeks	120·1	110·3	201·9
14 weeks	140·9	179·5	233·9
S.E. of single mean	4·3	6·6	8·1
Significant difference, *P* 0·05	None	20·1	27·5
(*d*) Level of high-temperature storage			
18° C.	125·1	132·0	195·3
21° C.	126·9	135·0	202·8
24° C.	128·7	128·3	210·0
S.E. of single mean	4·3	6·6	8·1
Significant difference	None	None	None

large sets produced a slightly larger yield than medium sets, and these again a larger yield than small sets. Thus as far as gross yield was concerned large-set size appeared on the average to be an advantage in spite of bolting. The interactions discussed below show that this was not always the case, and the poor quality of bolted bulbs must also be remembered. The average effects of the different temperature levels were not sufficiently marked at any centre to be significant. The usual pronounced effect of high-temperature storage on yield was, however, shown by the average effects of duration, although treatment for 7 weeks was almost without effect except in Scotland. In general, conditions under which late ripening was the rule (Cockle Park and Auchincruive) tended to accentuate the effects of treatments which themselves caused ripening to be delayed.

Of the two-factor interactions, that between variety and duration of heat treatment was found significant at Wisley, between variety and level of high-temperature storage at both Cockle Park and Auchincruive and between variety and set size only at Auchincruive. These effects are set out in Table 23. The interactions between variety and duration (Table 23(*a*)), or between variety and temperature level (Table 23(*b*)), show, in general, that heat treatment considerably increased the yield from Red Wethersfield, but not from Ebenezer. Indeed, the higher storage temperature apparently *decreased* the yield for Ebenezer, but inspection of the

TABLE 23. *Experiment IV*, 1942–3. *Mean yield, g./plant. Two-factor interactions*

(*a*) Interaction of variety and duration of high-temperature storage

	Treatment for (weeks)		
	0	7	14
Wisley:			
Ebenezer	146·6	138·6	142·6
Red Wethersfield	92·7	101·8	139·2

S.E. of single mean = 6·1
Significant difference, P 0·05 = 17·4

(*b*) Interaction of variety and level of high-temperature storage

	Storage at		
	18° C.	21° C.	24° C.
Cockle Park:			
Ebenezer	178·3	177·8	144·3
Red Wethersfield	85·6	92·4	112·3

S.E. of single mean = 9·4
Significant difference, P 0·05 = 20·1

Auchincruive:			
Ebenezer	264·2	255·2	246·4
Red Wethersfield	126·4	150·3	173·8

S.E. of single mean = 11·5
Significant difference, P 0·05 = 34·9

(*c*) Interaction of variety and set size

	Large (4–7 g.)	Medium (2–4 g.)	Small (<2 g.)
Auchincruive:			
Ebenezer	280·1	243·8	241·8
Red Wethersfield	131·0	162·2	157·3

S.E. of single mean = 11·5
Significant difference, P 0·05 = 34·9

three-factor interaction variety × level × duration (Table 24) shows that this was mainly, if not entirely, due to fortuitous low values of the 24° C. × 0 week treatment for Ebenezer. This absence of effect of high temperature on yield of Ebenezer was explicable by the relative lack of bolting in this variety, but even so, it is unusual. Thus in Exp. I, the yield was doubled by 24° C. treatment (p. 476), in Exp. III a considerable although not quite significant increase was recorded (p. 483), and in other experiments at Woburn and Rothamsted similar results have been obtained. A further suggestion in this connexion arises out of Exp. V (below).

The data in Table 24 again demonstrate that where ripening is late, treatments which delay ripening have an enhanced effect upon yield. At Wisley, where ripening was very rapid, only Red Wethersfield showed an effect of high temperature, and that only when given for 14 weeks. At Cockle Park, a similar but larger effect was shown by Red Wethersfield, and there is an indication that 21° C. for 14 weeks increased the yield of Ebenezer. At Auchincruive even 7 weeks' heat

treatment appears to have had some effect for both varieties, and in Red Wethersfield a further 7 weeks' treatment caused a further increase in yield.

TABLE 24. *Experiment IV*, 1942–3. *Mean yield, g./plant. Three-factor interaction —variety and level and duration of high-temperature treatment*

	Duration (weeks)	Level 18° C.	21° C.	24° C.
Wisley:				
Ebenezer	0	146·5	156·9	136·6
	7	135·1	134·7	145·5
	14	149·8	142·2	135·1
Red Wethersfield	0	89·3	96·9	92·1
	7	103·0	97·8	104·4
	14	126·2	132·8	158·8
Cockle Park:				
Ebenezer	0	187·1	140·8	104·0
	7	140·8	152·1	134·2
	14	207·9	241·0	194·7
Red Wethersfield	0	66·2	54·8	81·3
	7	79·4	76·5	79·4
	14	111·5	145·5	176·7
Auchincruive:				
Ebenezer	0	236·3	241·9	203·2
	7	277·8	265·5	258·9
	14	278·8	258·9	276·9
Red Wethersfield	0	105·8	129·5	118·1
	7	135·1	131·4	141·8
	14	138·0	189·9	260·8

At Wisley and Cockle Park the yields in both varieties were in the order large > medium > small. At Auchincruive (Table 23 (*c*)), on the other hand, although this was true of Ebenezer, in Red Wethersfield the large sets gave a slightly smaller yield than either medium or small sets. This is almost certainly a result of the extra bolting—which occurred mainly in the large-size class, being sufficient, in Scotland, to depress the yield from large sets.

(*c*) *Ripening*. In this and the following experiments the ripening data collected were such as to allow bolted plants to be eliminated. Thus, treatment effects on the rate of ripening can be separated from those on bolting; it has been found that the scape prevents the foliage of bolted plants bending over. The data presented are, of course, not comparable with those for ripening in Exps. I, II and III, where separate counts of bolted and non-bolted plants were not available.

Unfortunately, no data on ripening were obtained from the Scottish experiment, and the figures given in Table 25 for Wisley and Cockle Park are themselves not strictly comparable, as nearly a month separates the dates on which the respective counts were made. This circumstance, however, reflects one effect of the different centres on ripening; the plants at Cockle Park, although counted later than at Wisley, were less ripe. Ripening was also later in Scotland, illustrating the primary

importance of earliness in northern districts. The average earliness of the varieties, as estimated, was not greatly different at Wisley (61% of the Red Wethersfield having bent over, compared with 66% of the Ebenezer plants), but at Cockle Park, Red Wethersfield appears distinctly earlier (33 and 22% respectively). If the figures for the control temperature treatments (i.e. '0 week duration' in Table 25) are compared, it is seen, however, that at Wisley, Ebenezer appears to be somewhat the earlier.

TABLE 25. *Experiment IV*, 1942–3. *Mean percentage of plants which had bent over by 13 July 1943 (Wisley) or 7 August 1943 (Cockle Park). Three-factor interaction—variety, and level and duration of high-temperature treatment*

	Duration (weeks)	Level 18° C.	21° C.	24° C.
Wisley:				
Ebenezer	0	81	72	78
	7	70	69	51
	14	50	66	54
Red Wethersfield	0	48	49	75
	7	55	64	74
	14	67	46	70
Cockle Park:				
Ebenezer	0	20	37	47
	7	19	21	18
	14	6	16	13
Red Wethersfield	0	52	44	70
	7	46	60	7
	14	3	5	8

The usual effect of high-temperature storage in delaying ripening is apparent at Cockle Park (compare the 0, 7 and 14 weeks' treatment in the table), but is almost absent at Wisley.

EXPERIMENT V

1942–3 (*Wisley*). *Set size and storage temperature*

Design and analysis

As in Exp. IV, a closer analysis of the most suitable high storage temperature was attempted. In each case 14 weeks' treatment was given from 23 November, and the *four temperatures* used were common storage at about 7° C. as control and the high temperatures 18, 21 and 24° C. (approximate). The *four set sizes* were large (4–7 g.), medium (2–4 g.) and small (< 2 g.), and in addition oversize (7–10 g.). The variety was Ebenezer, the sets being raised at Wisley. The sixteen treatments were replicated five times and laid out as randomized blocks making, in all, eighty plots each of fifteen plants. The spacing and method of planting were as in previous experiments.

Experimental technique and field notes

Experimental technique followed that of the previous experiment exactly. The sets were planted on 11 March.

Results

(*a*) *Bolting.* There was no bolting in the small-size class, and this treatment is, therefore, omitted from the discussion that follows. The means for the other size classes are shown in Table 26, flowering not being serious in any treatment. The differences between these treatments just reached significance when compared with the interaction size × temperature and were very highly significant tested against the random variance. The average storage temperature effect was also very highly significant tested against 'random' (Table 26).

TABLE 26. *Experiment V*, 1942–3 (*Wisley*). *Mean percentage bolting by 29 June* 1943. *Average effects of single factors*

Set size		Storage at	
Oversize (7–10 g.)	23·5	7° C.	31·5
Large (4–7 g.)	4·0	18° C.	3·2
Medium (2–4 g.)	0·5	21° C.	4·5
		24° C.	0·2

Significant differences*: Oversize > large > medium. 7 > 18 and 21 > 24° C.

* When compared with random variation, but not with interactions.

The interaction effect (Table 27) was very highly significant; in the medium-size class all treatments showed a negligible amount of bolting, but in the large and oversize classes bolting was substantially reduced by a storage temperature of 18 or 21° C., and prevented by a temperature of 24° C. It is clearly shown that the highest temperature is needed to control bolting in larger sets.

TABLE 27. *Experiment V*, 1942–3 (*Wisley*). *Mean percentage bolting by 29 June* 1943. *Two-factor interaction—set size and storage temperature*

	Storage at			
Set size	7° C.	18° C.	21° C.	24° C.
Oversize (7–10 g.)	80·7	14·0	19·1	0·6
Large (4–7 g.)	28·8	1·1	1·5	0·0
Medium (2–4 g.)	1·1	0·5	0·5	0·0

(*b*) *Yield.* The average effects on yield both of set size and of storage temperature were highly significant but not the interaction effect of these factors. The yields (Table 28) steadily increased the larger the sets used. Ebenezer is known to be a variety of little inherent tendency to bolt (Holdsworth, 1945) but the very low

TABLE 28. *Experiment V*, 1942–3 (*Wisley*). *Mean yield, g./plant. Average effects of single factors*

Set size		Storage at	
Oversize (7–10 g.)	171·2	7° C.	112·3
Large (4–7 g.)	141·8	18° C.	131·5
Medium (2–4 g.)	112·3	21° C.	147·1
Small (< 2 g.)	91·3	24° C.	125·6

S.E. of a single mean. = 5·0

Significant difference between two means, P 0·05 = 14·2

average bolting, and consequent high yields, in the oversize class are remarkable. The means for the storage-temperature treatments show that the highest storage temperature (24° C.) caused a reduction in yield compared with 21° C.; in fact, the yield from the sets treated at 24° C. just fails to differ significantly from that of the controls. The interaction means show that the poor yields given by the 24° C. treatment are most pronounced in the smaller set-size classes, where the increased yield caused by high temperature is probably purely an effect of the delay of ripening. Thus it seems that bolting is controlled most by very high temperature, but time of ripening is affected most by medium high temperature.

(*c*) *Ripening*. The treatment effects upon ripening were estimated from the number of plants, excluding those bolted, which had bent over on 13 July. The count was made rather late, so that a considerable proportion of plots in which all were bent over occurred in all treatments. Treatment differences, therefore, were small but are fairly consistent. The means are shown in Table 29. Earliness was

TABLE 29. *Experiment V*, 1942–3 (*Wisley*). *Mean percentage of plants which had bent over by* 13 *July* 1943. *Average effects of single factors and two-factor interaction*

(*a*) Single factors

Set size		Storage at	
Oversize (7–10 g.)	85	7° C.	88
Large (4–7 g.)	84	14° C.	79
Medium (2–4 g.)	81	21° C.	75
Small (< 2 g.)	77	24° C.	85

(*b*) Interaction of set size and storage temperature

	Storage at			
Set size	7° C.	18° C.	21° C.	24° C.
Oversize (7–10 g.)	100	71	81	89
Large (4–7 g.)	88	83	79	87
Medium (2–4 g.)	86	74	75	90
Small (< 2 g.)	79	88	67	74

found to increase with increasing set size (cf. Holdsworth, 1945). High-temperature storage again delayed ripening, although the highest storage-temperature treatment had only a slight effect, confirming the suggestion made above that the maximum delay of bulbing follows a storage temperature of about 20° C. Small but not very consistent effects of the same kind are suggested by the ripening data for Exp. IV (Table 25).

EXPERIMENT VI

1942–3 (*Wisley*). *Variety, set size and nitrogen manuring (time and rate of application)*

Design and analysis

Sets of *Ebenezer and Brown Globe*, produced at Wisley in 1942, were used for this experiment. The Brown Globe sets were the larger, although they came from a later sowing. Thus the *two size classes* (large and small) do not correspond exactly, being

2–10 and 0–2 g. for Ebenezer and 4–10 and 0–4 g. for Brown Globe. The nitrogen manurial treatments were considered as *two times of application* (early, 2 April and late, 30 April), each at *four rates* (none, $2\frac{1}{3}$, $4\frac{2}{3}$ and 7 cwt. sodium nitrate/acre). This made thirty-two treatments in all ($2^3 \times 4$) and the whole was duplicated to give a total of sixty-four plots, each of fifteen plants. Each replication was divided into two blocks by confounding the interaction 'variety × set size × time of application × cubic term of the rate of application of nitrogen'.

Experimental technique and field notes

The sets were planted on 11 March. The method of fertilizer application was as described for Exp. I (p. 474). Throughout the season, no pronounced differences of colour or habit due to additional nitrogen were observed. The plots all received a basal dressing of 5 cwt. superphosphate and 5 cwt. potassium chloride earlier in the spring.

Results

(*a*) *Bolting*. When tested against the random variation all the treatments gave significant average effects excepting rate of nitrogen application. Those for variety and set size reached the P 0·001 level of significance (Table 30). The effect of set

TABLE 30. *Experiment VI*, 1942–3 (*Wisley*). *Mean percentage bolting by 1 July 1943. Average effects of single factors*

Ebenezer	3·5	Early nitrogen application	26·1
Brown Globe	42·8	Late nitrogen application	22·1
		Large sets	40·5
		Small sets	4·6

Significant differences: Ebenezer < Brown Globe. Early > late, large > small*.

* When compared with random variation, but not with interactions.

size was of the usual kind, and Ebenezer was again shown to have little tendency to bolt. The average effect of time of application of nitrogen is curious in the absence of a significant effect of nitrogen application itself. If the effect were real it would be expected that a nitrogen effect would show in one of the interactions involving nitrogen dosage. In fact only the variety × set size two-factor interaction (Table 31)

TABLE 31. *Experiment VI*, 1942–3 (*Wisley*). *Mean percentage bolting by 1 July 1943. Two-factor interaction—variety and set size*

	Large sets	Small sets
Ebenezer	13·6	0·0
Brown Globe	70·7	17·1

had a statistically significant effect. The means demonstrate a larger difference in bolting between the set-size classes of Brown Globe than of Ebenezer—an effect which may perhaps be ascribed partly to the differences of the size classes in the two varieties.

(*b*) *Yield.* The nitrogen effects both of rate and time of application were quite negligible, as also were their interactions. The average effects of variety and set size, although large, were not significant if compared with their interaction, the means being: for Ebenezer 116·8 g./plant, for Brown Globe 97·2 g./plant, and for large sets 117·9 g./plant, for small 96·1 g./plant. The means for the highly significant interaction set size × variety are set out in Table 32. The difference in yield of the

TABLE 32. *Experiment VI*, 1942–3 (*Wisley*). *Mean yield, g./plant. Two-factor interaction—variety and set size*

	Large sets	Small sets
Ebenezer	137·2	96·1
Brown Globe	98·4	95·8
S.E. of a single mean		= 5·9
Significant difference between two means, P 0·05 = 16·8		

two set sizes was much greater in Ebenezer than in Brown Globe, presumably because in the latter, yield from the large sets was appreciably diminished by bolting.

(*c*) *Ripening.* The treatment effects on ripening have been estimated from the percentage of non-bolting plants which had bent over by 13 July, as set out in Table 33. The average ripeness for each of the varieties was practically the same,

TABLE 33. *Experiment VI*, 1942–3 (*Wisley*). *Mean percentage of plants which had bent over by 13 July 1943. Average effects of single factors and two-factor interaction*

(*a*) Single factors

Ebenezer	44	Early nitrogen application	40
Brown Globe	45	Late nitrogen application	50

Nitrogen dressing: Nil 45; Single 41; Double 50; Treble 43

(*b*) Interaction of variety and set size

	Large sets	Small sets
Ebenezer	56	32
Brown Globe	48	42

but whereas in the plants from large sets, Ebenezer was earlier than Brown Globe, for small sets the converse held. This interaction may have been due to the difference in the size classes for the two varieties, since for each variety the plants from small sets are later. The data for Brown Globe show an effect of nitrogen manuring such that nitrogen delayed ripening when given early but accelerated it, especially when given at the rate of $4\frac{2}{3}$ cwt./acre, if applied late. For Ebenezer there was little effect.

Experiment VII

1942–3 (*Wisley*). *Variety, set size and nitrogen manuring (rate of application and source of nitrogen)*

Design and analysis

The design of this experiment was very similar to that of Exp. VI, but compared ammonium with nitrate nitrogen. Thus the factorial design was constructed of the factors: *two varieties* (Ebenezer and Danvers Yellow Globe) × *two sizes* of set (large and small) × *two sources of nitrogen* (ammonium sulphate and nitrochalk) × *four rates of application* (none, 42·4, 84·8 and 127·2 lb. of nitrogen/acre). As Ebenezer produces much smaller sets than Danvers Yellow Globe for the same rate of seed sowing, it was necessary to use sets from a much later sowing of the latter to obtain comparable size classes. Even so the correspondence was only approximate: large was 2–10 g. for Ebenezer and 5–10 g. for Danvers Yellow Globe; small 0–2 and 0–5 g. respectively. As in Exp. VI, the interaction variety × set size × source of nitrogen × cubic term of the rate of application was confounded, and the whole experiment duplicated so that there were sixteen plots in each of four blocks. There were fifteen plants per plot.

Experimental technique and field notes

Methods and date of planting were as in the preceding experiment.

Results

(*a*) *Bolting.* The average effects of set size and of variety (Table 34) were both very highly significant statistically when tested against the random variance.

Table 34. *Experiment VII*, 1942–3 (*Wisley*). *Mean percentage bolting by 1 July 1943. Average effects of single factors*

Ebenezer	5·5	Large sets	47·8
Danvers Yellow Globe	43·9	Small sets	3·8

Significant differences*: Ebenezer < Danvers Yellow Globe. Large > small.

* When compared with random variation, but not with interactions.

Neither of the nitrogen treatments, however, was significant and their effects were negligible. None of the two-factor interactions involving nitrogen manuring produced a significant effect.

The means for the set size and variety interaction are set out in Table 35. It is again seen that the plants of variety Ebenezer showed little tendency to bolt. The effect of set size is smaller in Ebenezer than in Danvers Yellow Globe, but these

Table 35. *Experiment VII*, 1942–3 (*Wisley*). *Mean percentage bolting by 1 July 1943. Two-factor interaction—variety and set size*

	Large sets	Small sets
Ebenezer	20·6	0·0
Danvers Yellow Globe	75·7	14·6

interaction data are again of uncertain value in view of the initial differences between the corresponding size ranges in each variety.

(*b*) *Yield.* None of the factors produced a significant average effect on yield, and the variances for nitrogen manuring, both dosage and type of manure, were negligible. The effects for variety and set size were large, although, as stated above, not significant when tested against their interaction. The interaction between set size and variety was very highly significant and the yields are shown in Table 36(*a*), where it is seen that the differences between the yields of the two size classes were much greater in Ebenezer than in Danvers Yellow Globe, as in Exp. VI for Ebenezer and Brown Globe, and presumably for the same reason.

TABLE 36. *Experiment VII*, 1942–3 (*Wisley*). *Mean yield, g./plant. Two-factor interactions*

(*a*) Interaction of variety and set size

	Large sets	Small sets
Ebenezer	148·8	107·7
Danvers Yellow Globe	100·1	84·8

S.E. of a single mean = 4·3
Significant difference between two means, P 0·05 = 12·3

(*b*) Interaction of set size and nitrogen dressing

Nitrogen (lb./acre) ...	Nil	42·4	84·8	127·2
Large sets	134·7	114·0	123·0	126·7
Small sets	87·3	102·9	106·9	88·2

S.E. of a single mean = 6·1
Significant difference between two means, P 0·05 = 17·4

The only other appreciable two-factor interaction was that between set size and nitrogen dosage, which was significant at the P 0·05 level (Table 36(*b*)). Nitrogen manuring depressed the yield of plants from large sets but the yield from small sets was increased significantly by a double dressing and possibly by a single dressing.

(*c*) *Ripening.* Table 37 indicates that Ebenezer is earlier than Danvers Yellow Globe, and that, as in other experiments, the bulbs from large sets ripen before those from small sets. In Danvers Yellow Globe, the effect of set size on ripening was much smaller

TABLE 37. *Experiment VII*, 1942–3 (*Wisley*). *Mean percentage of plants which had bent over by* 15 *July* 1943. *Average effects of single factors and two-factor interactions*

(*a*) Single factors

Ebenezer	67
Danvers Yellow Globe	45

(*b*) Interaction of variety and set size

	Large sets	Small sets
Ebenezer	70	63
Danvers Yellow Globe	48	44

(*c*) Interaction of nitrogen source and dressing

Nitrogen (lb./acre) ...	Nil	42·4	84·8	127·2
Ammonium sulphate	62	50	49	51
Nitrochalk	61	58	56	64

than in Ebenezer, but this again may have been due to the different size classes used. There is evidence for the view that nitrogen manuring delays ripening, ammonium sulphate being more effective than nitrochalk and the records of Scully, Parker & Borthwick (1945) on the effect of nitrogen on earliness of bulbing may be cited.

DISCUSSION

Much of the earlier work on onion physiology has been summarized by one of us elsewhere (Heath, 1945), and familiarity with this paper will be assumed.

The experiments collected here have investigated the effects of variety (Exps. II–V), of set size (Exps. I–VII), of storage temperature (Exps. I–V) and of time of planting (Exp. I), and confirm data presented for these factors and their interactions in earlier published work. In addition, data from experiments (I, II, VI and VII), in which nitrogen manurial factors also were included, are presented.

It was found that some varieties, e.g. Ebenezer, do not show a great incidence of bolting unless very large sets are used; this indicates the possibility of selecting varieties for freedom from bolting. Again, it has been found without exception that the larger the size of set used the greater is the incidence of bolting. The effect of set size on yield is complicated by the effect of bolting which depresses yield, otherwise large sets have been found to give consistently higher yields than small sets. Thus the selection of a low-bolting strain enables advantage to be taken of the high-yielding qualities and general hardiness of plants grown from large sets.

The effects of storage temperature both confirmed earlier work and provided some additional information. Thus it was confirmed that high-temperature storage could adequately control bolting in all but very large sets when applied for at least 14 weeks during the winter. On the other hand, treatment for the first 7 weeks only was without effect except in the low-bolting variety Ebenezer. The reduction of bolting previously found with 8 weeks' treatment (Heath, 1943*a*) was obtained with a much higher temperature (30° C.) than any used in the present experiments. The level of high temperature necessary to control bolting depends upon both the inherent tendency to bolt of the strain used and the set size (Table 38); for a given variety and size, bolting tends to be progressively reduced as the temperature is increased. This is evidence for the hypothesis that the qualitative change from a vegetative to a reproductive condition of the growing point (Heath & Mathur, 1944) is caused by the presence of more than a critical quantity of some substance which is destroyed by heat at a rate which increases with temperature. For the variety Ebenezer, 18° C. for 14 weeks controlled the bolting of plants from sets up to 7 g. weight, but for sets up to 10 g., 24° C. treatment was needed. (One aberrant result was obtained with Ebenezer in Exp. I, where 4–7 g. sets gave 15% bolting after 14 weeks at 24° C.) In the high-bolting variety Red Wethersfield, bolting among the plants from 4 to 7 g. sets, although greatly reduced by 24° C. treatment for 14 weeks, was still serious at the northern centres (43–47%) and appreciable at

Wisley (11%). The control treatment (7° C.) gave 69–83% bolting. (In an additional experiment carried out at Wisley in 1942 bolting was completely prevented in sets up to 16 g. in some varieties, by 14 weeks' treatment at 24° C.) Even in the size

TABLE 38. *Summary. Mean percentage bolting. Average effects of storage temperature (14 weeks' treatment)*

Experiment	Set size (g.)	Storage temperature 0° C.	7° C.	18° C.	21° C.	24° C.
	(*a*) Ebenezer					
I. Wisley	4–7	44	45	—	—	15
	2–4	10	10	—	—	0
	0–2	0	2	—	—	0
III. Wisley	4–7	25	34	—	—	0
	2–4	2	3	—	—	0
	0–2	0	0	—	—	0
IV. Wisley	4–7	—	2	12	0	0
	2–4	—	1	0	0	0
	0–2	—	0	0	0	0
Cockle Park	4–7	—	19	0	0	0
	2–4	—	2	0	0	0
	0–2	—	0	0	0	0
Auchincruive	4–7	—	16	0	0	0
	2–4	—	2	0	0	0
	0–2	—	0	0	0	0
V. Wisley	7–10	—	81	14	19	0·6
	4–7	—	29	1	2	0
	2–4	—	1	0·5	0·5	0
	0–2	—	0	0	0	0
	(*b*) Red Wethersfield					
IV. Wisley	4–7	—	69	67	62	11
	2–4	—	35	25	42	10
	0–2	—	4	11	2	0
Cockle Park	4–7	—	83	67	46	43
	2–4	—	48	42	38	33
	0–2	—	3	0	7	0
Auchincruive	4–7	—	79	67	67	47
	2–4	—	44	40	27	7
	0–2	—	18	7	0	0
	(*c*) Other varieties					
II. Bedfordshire Champion (Wisley)	7–10	79	75	—	—	20
	4–7	27	25	—	—	2
	2–4	2	2	—	—	0
Best of All (Wisley)	7–10	56	69	—	—	4
	4–7	25	34	—	—	2
	2–4	8	11	—	—	0

class 2–4 g. bolting of Red Wethersfield at Cockle Park was only reduced by 24° C. treatment to 33%. This very much heavier bolting at the northern centres may well have been due to delayed bulbing allowing the emergence of inflorescences initiated after planting. It is therefore somewhat doubtful whether even high temperatures,

such as that used by Heath (1943*a*), would be effective under these circumstances, although evidence was obtained (Heath & Mathur, 1944) for considerable after-effects of high-temperature storage in preventing inflorescence initiation subsequent to treatment. As long as a low-bolting strain is used in northern districts, however, a storage treatment of about 20° C. for 14 weeks should be adequate for large sets (4–7 g.), and for smaller sets no special treatment should be needed. The latter alternative is probably preferable so that delay of ripening may be avoided.

Low-temperature storage at 0° C. for 14 weeks (Exps. I–III) reduced bolting appreciably in the case of the smaller sets, thus confirming an earlier finding (Heath, 1943*a*). The effect is, however, too small to have practical value.

Pronounced effects of high-temperature storage for 14 weeks or longer in delaying ripening have been found, but in Exp. V, in which a range of high-temperature treatments was tested, the highest temperature (24° C.) had less effect than the others (18 and 21° C.). Some suggestion of a similar effect on Ebenezer was found in Exp. IV. These differences between the effects of the various high temperatures on ripening were reflected in the yields. In general, high-temperature storage results in remarkably increased yields, sometimes 400 % or more (Heath, 1943*a*; Blaauw *et al.* 1941, 1944), and, in the absence of an effect on bolting, this is thought to be wholly due to the delayed bulbing and ripening resulting in more leaves and an extended period of growth (Heath, 1943*b*). Therefore, if bolting is not heavy, a maximum effect on yield may be expected (as in Exp. V) after storage at temperatures somewhat below 24° C. and in the region of 20° C. Blaauw *et al.* (1944) record likewise that very high storage temperatures (28–31° C.) slightly depress the yield compared with $25\frac{1}{2}$° C., although in their paper this is not expressly related to an effect on the rate of ripening. Where the incidence of bolting is high (as in the experiments of Blaauw and others and for Red Wethersfield in Exp. IV) the greater effect of the highest temperatures in controlling bolting may be expected to cause the largest yields after treatment at temperatures above 20° C. In Exp. II an interaction was found between set size and storage temperature. The tendency of large sets to produce larger yields was masked except where bolting was prevented by high temperature. Similar effects were found in Exp. III (variety Ebenezer) and in Exp. IV (Red Wethersfield) at Wisley.

It has been found in Exp. IV that under conditions where ripening tends in any case to be late, as in Scotland and northern England, the effect of heat treatment in delaying ripening and so increasing yield is much enhanced; conversely, where conditions promote rapid ripening, as at Wisley, the effect may be almost absent. These facts emphasize the undesirability of heat treatment for sets to be used in the north, where early ripening is most needed, and may also explain why the effect of high-temperature storage on ripening and yield (other than by the control of bolting) has not been noted in U.S.A. where ripening is presumably rapid. In this connexion it must be noted, however, that in Exp. IV the planting date at Wisley was almost a month earlier than at the northern centres, so that the data are not strictly comparable.

No effect of low-temperature storage on ripening or yield has been found in these experiments, and the results are thus in accord with other published work.

The effects of late planting of sets, described in Exp. I, confirm those found by Holdsworth (1945), which showed that late planting reduces the incidence of bolting, presumably because the onset of bulbing suppresses flower emergence and bulbing occurs rapidly in the long-day and high-temperature conditions of summer. For the same reason, viz. that bulbing occurs after a shorter growing period, late planting has the disadvantage that the bulbs harvested are smaller, the reduction in yield being considerable (a month's delay in planting in Exp. I depressed the average yield by 29%). Thus the increase of yield caused by early planting was greater when bolting was eliminated by the use of high-temperature storage, i.e. there was an interaction effect of planting date and storage temperature on yield.

As in many plants an important effect of nitrogen manuring is to increase total leaf area and prolong the life of the leaves, it was considered that the application of nitrogenous manure to late-planted sets might prevent the depression of yield usually produced by early bulbing and ripening. The effect of nitrogen on yield has, however, proved very small in all the experiments (I, II, VI and VII) where this factor has been investigated. It is not surprising that there should be no direct effect of nitrogen on bolting (the apparently significant effect of early versus late application of nitrogen in Exp. VI was almost certainly fictitious), but if nitrogen had had any appreciable effect on the time of bulbing, the emergence of inflorescences in the field might have been affected (Heath & Holdsworth, 1943) and this should have been reflected in the bolting counts. In fact, small effects of nitrogen manuring on the rate of ripening were apparent in Exps. VI and VII and the method of estimating ripening used, viz. proportion of plants bending over at the neck is known (Holdsworth & Heath, 1945) to be a measure of the earliness of bulbing.* In the 1942 experiments, however, no such effect was shown, and the effects found in 1943 were too small to have had any appreciable effect on the emergence of inflorescences.

In Exps. I and VII small effects of nitrogen manuring on yield appeared in interaction effects—in the interaction nitrogen dosage × set planting date in Exp. I, and nitrogen dosage × set size in Exp. VII. In Exp. I a moderate dressing of nitrogen increased the yield but only where given to the early planting. In Exp. VII yield was increased where the dressing was applied to small sets but decreased when the application was to large sets. The absence of appreciable effect on late-planted sets is to be expected if nitrogen causes increased yields by increasing leaf growth, as the number of leaves which could be affected would be reduced because of the rapid onset of bulbing. On the other hand, it is difficult to find an explanation for the reduced yield found when nitrogen manure was applied to the plants from large

* Numbers of green leaves remaining at harvest or numbers of plants with green leaves remaining at harvest (Exps. I and II) give a better estimate of the rate of ripening, although earliness of bulbing is also involved.

sets of Exp. VII. It is clear, in any case, that these effects of nitrogen, even if real, are too small to serve as a practical method of compensating for the loss of yield occasioned by late planting.

The question as to why onions grown from sets have shown a negligible response to nitrogen cannot be answered from the data collected here. The site at Wisley was expected to provide only a moderate supply of nitrogen for an ordinary crop. A pot experiment (Heath & Holdsworth, unpublished) carried out at the Research Institute in 1943, with very nitrogen-deficient soil, also failed to show any appreciable response to nitrogen either in yield or nitrogen content, nor did the plants show any differences in colour. It is therefore probable either that the onion plant is efficient in tapping sources of nitrogen in the soil which are generally considered to be 'unavailable' or that sufficient nitrogen is carried over from one season to the next in the stored set. In the latter case normal response to nitrogen manuring would be expected on onion plants grown from seed.

A point of interest in the experiments described in this paper is that small bulbs picked out from commercial crops of large onions (Exps. II and III) have behaved in a manner essentially similar to sets of the same size specially grown as such (Exps. I, III, IV, V, VI and VII). It is commonly held that the former class is very much more liable to bolt. It is reasonable to suppose, however, that the smallest onions in a commercial crop have been produced by the operation of the same conditions, i.e. late germination and crowding, as are imposed during set production (Holdsworth, 1945; Tincker, Brown, Heath & Holdsworth, 1945).

All the practical experimentation has been undertaken by M. A. H. Tincker, with the assistance at Wisley of F. C. Brown, at Auchincruive of Dr B. T. Cromwell and at Cockle Park of Dr F. T. Bennett; O. V. S. Heath is responsible for the design of the experiments and with the assistance of M. Holdsworth for the analysis of the data. Grateful acknowledgement is made of the co-operation so freely given.

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THE BEHAVIOUR OF SOME NATURALLY OCCURRING STRAINS OF POTATO VIRUS *Y*

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(With Plate 13)

Potato virus *Y* was obtained from field crops of potatoes in many strains which differed widely in virulence and caused diseases in the variety Majestic ranging from severe leaf-drop streak to mild mosaic. The symptoms caused by these strains in seven potato varieties and tobacco are described and compared with those caused by the serologically related potato virus *C*. No changes were noted in the behaviour of any of the strains over three years, during which they were transmitted to many different plants.

Potato virus *C* was not transmitted by *Myzus persicae*, the most efficient vector of other strains of virus *Y*. Nor was virus *C* transmitted by eleven other species of aphides, eight of which transmitted virus *Y*. The efficiency with which different species acted as vectors of virus *Y* varied greatly, and it is suggested that in some species only occasional individuals can transmit.

Possible mechanisms for the evolution of viruses *C* and *Y* are indicated, and the effects of changes in virus, vector, and host on the prevalence of insect-transmitted viruses are discussed.

In his original account of the aphis-transmitted potato virus *Y*, Smith (1931) showed that it caused different symptoms in different potato varieties. Its most characteristic behaviour, however, was to cause a necrotic disease, leaf-drop streak, in the first year of infection and a non-necrotic disease, rugose (severe) mosaic, in subsequent years, that is, in plants raised from infected tubers. Smith isolated virus *Y* from six distinct sources and noted no differences between the behaviour of his different isolates. He commented on the apparent stability of the virus and contrasted it in this respect with potato virus *X*, of which his different isolates varied strikingly in the type of lesions they produced in tobacco plants. Smith concluded that most of the potato-mosaic diseases in England were caused by potato viruses *X* and *Y*, acting either alone or in combination, and that the range of symptoms encountered in these diseases resulted from variations in the strain of virus *X* rather than in virus *Y*.

Most plant viruses that have been studied in any detail have been found to exist in strains that can be differentiated by the type or severity of symptoms they produce, and potato virus *Y* seems to be no exception. Since 1931 there have been a number of suggestions that it may be related to other viruses and that it occurs in strains differing in their virulence towards potato and tobacco, but there has been little detailed work on the problem. The claims that virus *Y* is related to cucumber-mosaic virus (Chester, 1937) and potato virus *A* (Hansen, 1937) have not been

confirmed (Bawden & Sheffield, 1944), but it has been established that it is related to potato virus *C* (Cockerham, 1943; Bawden & Sheffield, 1944; Bald & Norris, 1945). Salaman (1937) obtained virus *Y* from *Schizanthus retusus* in a form which produced unusually mild symptoms in tobacco plants, and he stated that still less virulent variants could be produced by keeping infected tobacco roots in sterile nutrient solution for a week at temperatures above 22° C. These variants produced only slight symptoms in tobacco and a reduced form of leaf-drop streak in the potato varieties President and Up-to-Date, but they were not observed for long enough to assess their constancy. Köhler (1934, 1937) also described attenuated strains of virus *Y*, but Salaman (1937) considered that one at least was potato virus *A*. Smith & Dennis (1940) and Nobrega & Silberschmidt (1944) have described necrotic diseases of tobacco plants, which they attribute to unusually virulent strains of virus *Y*, but neither workers produce convincing evidence for relating their viruses to virus *Y*.

The probability that virus *Y* occurs naturally in a number of strains is suggested by the range in symptoms encountered in field crops affected with mosaic diseases. In varieties such as Majestic, which tolerate most strains of virus *X* and are usually infected with it, the differences could be caused by the presence of different strains of virus *X*, as suggested by Smith (1931), but comparable differences are also seen in varieties such as King Edward, which are hypersensitive to most strains of virus *X* and are rarely found naturally infected with it. The work described in the present paper was undertaken to determine the cause of these differences. It shows that potato virus *Y* as it occurs in potato crops is not a single entity, but comprises many strains that differ in their virulence and can be distinguished by constant differences in the symptoms they produce.

Material and methods

From field examinations in 1943 of King Edward and Majestic potato crops in various parts of England, plants were selected which showed mosaic diseases of different degrees of severity and which were thought possibly to be infected with potato virus *Y*. Leaf samples were taken, the sap expressed and inoculations made to tobacco, var. White Burley, and *Datura stramonium*, which are convenient indicator plants for viruses *X* and *Y* (Smith, 1931). Of the sixteen Majestic plants tested, four contained only potato virus *X*, strains that gave severe necrosis and ringspot in tobacco and *D. stramonium*. When transmitted to healthy Majestic plants, these strains produced a severe mosaic with necrotic spotting, leaf deformity and some shrivelling and falling of the lower leaves. The primary symptoms could be distinguished fairly readily from leaf-drop streak caused by virus *Y* because of the absence of elongated veinal necroses along the undersides of the leaves. When plants were raised from infected tubers, however, symptoms were very similar to those of rugose mosaic caused by virus *Y*, and differentiation was more difficult, especially early in the season. As the season advanced and the temperature

increased, plants infected with these strains of virus *X* tended to recover and the later formed leaves showed less deformity and mosaic, whereas there is no such effect with plants infected with virus *Y*. Of the remaining twelve Majestic plants, one contained virus *Y* only and the others contained both viruses *X* and *Y*. Of the fifteen King Edward plants tested, fourteen gave no reaction on *D. stramonium* and gave only virus *Y*, but the fifteenth contained a strain of virus *X*. This was not studied in any detail, but it was presumably a strain similar to X^a or X^b (Bawden & Sheffield, 1944), which do not produce top-necrosis in King Edward.

From the inoculated tobacco plants showing symptoms suggesting infection with virus *Y* or with *X* and *Y* together, transmissions were made by means of *Myzus persicae* Sulz. to further healthy tobacco seedlings. The aphides were starved for 4 hr. before being fed for from 3 to 5 min. on the infected plants; after this they were transferred immediately to the test plants, on which they were left for 24 hr. before being killed by fumigation. Watson & Roberts (1939) have shown that this treatment is the most effective for transmitting virus *Y*, and, unless otherwise stated, it has been used throughout this work. All the isolates obtained from the field were readily transmitted by *M. persicae*, and tobacco plants infected with single aphides were used as the sources of inoculum for subsequent work.

To check the identity of the aphis-transmitted viruses still further, all those obtained from the field were tested for their serological relationship with a derivative of Smith's original culture of virus *Y*. Antisera were prepared against this virus by injecting rabbits intraperitoneally with sap from infected tobacco plants which was clarified by freezing, thawing, and centrifuging at 6000 r.p.m. Six injections each of 5 ml. of sap were given at weekly intervals and the rabbits were bled 10 days after the last injection. Clarified sap from plants infected with all the isolates precipitated specifically with this antiserum, showing that they are all strains of virus *Y*.

In addition to the twenty-six isolates of virus *Y* from the field and the stock culture, comparisons were also made with the serologically related potato virus *C*, obtained from an Edgecote Purple potato plant kindly supplied by Mr R. J. Scott.

Most inoculations were made by rubbing the leaves of healthy plants with the forefinger wet with infective sap, but in some transmissions the rubbed leaves were first lightly dusted with kieselguhr or 400-mesh carborundum, a treatment that facilitates transmission. The ultimate reactions of most plants were the same whether they were infected by aphides or by inoculation with or without the use of abrasives, but the different methods of infection initially produced differences. These were most striking with host-virus combinations that gave necrotic symptoms, for example, Majestic with a strain of virus *Y* that gives leaf-drop streak in the first year of infection. Infection with aphides rarely gives any definite local lesions, though some necrosis of the veins sometimes occurs on the infected leaf. Local necrotic lesions are obtained by inoculation, but there are many more when an abrasive is used. With an abrasive also, systemic symptoms appear several days earlier than with the other methods, the necrosis of the lower leaves is more

extensive, and the mosaic symptoms of the upper leaves appear earlier and are more severe. It seems that the time taken for the development of systemic symptoms and their initial severity depends on the size of the infecting dose of virus. The amount of virus introduced is much increased by using an abrasive, so that opportunity for moving out of the inoculated leaf occurs sooner than with other methods of infection, and the plant gets more virus distributed through it at an earlier stage of its development. With Katahdin, the final symptoms were sometimes influenced by the method of infection. Inoculation with an abrasive almost invariably produced a systemic infection with all the strains used, but with some strains inoculation without abrasive or by aphides sometimes gave necrosis only in the inoculated leaf or an incompletely systemic infection restricted to scattered necrotic lesions on a few leaves.

The symptoms produced by different strains of virus Y

All the isolates of virus *Y* and virus *C* produced symptoms of the same general type in tobacco, an initial 'vein-clearing' succeeded by 'vein-banding'. Seven to ten days after infection, the time depending on the season, size of plant, method of infection and the strain, there is a slight crinkling of the young leaves and their veins become picked out by the tissue around them becoming a lighter green or yellow. This mottle fades and is succeeded by pallor of the interveinal areas accompanied by the formation of dark green bands of tissue along the veins. Although all the isolates gave this sequence of symptoms, there were constant differences between the severity of the disease caused by different isolates. Whereas some produced bright vein-clearing and vein-banding, with considerable waving and crinkling of the leaves and stunting of the plants, others produced milder leaf symptoms and had less effect on the growth of the plants.

Differences between the behaviour of the individual isolates were still more obvious when they were transmitted to the potato varieties Majestic, Doon Star, Gladstone and Arran Pilot, for with each variety there was a wide range in the type and severity of symptoms produced by the different isolates. In Majestic, for example, some produced necrotic local lesions and severe leaf-drop streak, all the middle and lower leaves becoming necrotic and soon falling, while the few remaining upper leaves showed a bright mottle and considerable deformity; other isolates produced fewer and less severe necroses, only the lower leaves falling, while the middle and upper were mottled and crinkled; still others produced no necroses, but only mosaic symptoms of varying severity (Pl. 13, fig. 1). In Arran Pilot none of the isolates caused necrotic symptoms, but the mosaic and rugosity caused by different isolates varied from severe to negligible. Although there were such wide varietal differences in reaction, the relative virulence of the different isolates seemed to be much the same for all four varieties, that is, the strains that caused the most and the least severe symptoms in Majestic also caused the most and the least severe symptoms in the other three varieties. This contrasts with the behaviour of strains of virus *X*,

many of which are virulent towards some varieties but avirulent towards others, so that a knowledge of the type of disease caused in one potato variety or in tobacco is little guide as to what its effects will be in another. However, that this is not a fundamental difference between the two viruses is indicated from the results described below with potato virus *C*. Some of the isolates of virus *Y* from the field duplicated each other's behaviour, and as it was impossible to study all in sufficient detail to see whether they were identical or not, twenty were discarded. The seven retained for further work were the two most virulent, three intermediate and the two least virulent. These will be referred to as *Y* 1, *Y* 2, etc., *Y* 1 being the isolate causing the most severe disease in Majestic and *Y* 7 the least severe. *Y* 1 is the stock culture derived from Smith's original isolate, and it is of some interest that we found no strain more virulent than this.

The differences between the symptoms caused by these different isolates are amply adequate for regarding the viruses as distinct strains. Their reactions have been observed over 3 years without any changes being noted. They have been transmitted to new plants on many occasions, by aphides and by inoculation, and have been propagated in both potato and tobacco plants, but their behaviour on a range of potato varieties has remained constant. Each year healthy plants of the varieties Majestic, King Edward, Doon Star, Arran Pilot, Arran Banner, Gladstone and Katahdin have been inoculated in the spring to observe the primary symptoms. The plants were raised in an insect-proof glasshouse, and at least two of each variety were infected when they were about 10 in. high. Tubers from these plants were saved and grown in succeeding years, both under glass and in the open, to determine the type and constancy of the secondary symptoms. A summary of the symptoms produced by four of the strains, two extremes and two intermediates, on the seven varieties is given in Table 1 (Pl. 13, figs. 2, 3). There is, of course, no reason to believe that there are not further strains of virus *Y* that might cause other symptoms than those recorded.

The potato varieties were also infected with potato virus *C* to compare its behaviour with that of the other strains. It produces a wider range of symptoms than any of the others, and its virulence relative to the others depends on the host plant used. In Majestic, King Edward and Doon Star, virus *C* causes a more severe disease than any of the other strains tested. These varieties are hypersensitive to virus *C*; if infected by inoculation only necrotic local lesions result, whereas if infected by grafting the plants die from top-necrosis. The other four varieties and tobacco all become systemically infected by inoculation, and in these the diseases produced are similar to those caused by some of the other strains of virus *Y*. Arran Pilot and Arran Banner react only with mosaic symptoms, comparable in intensity with those produced by *Y* 5 and *Y* 6. Gladstone and Katahdin give some necrotic local lesions, followed by systemic symptoms consisting of veinal necroses and leaf-drop streak of the lower leaves and a mosaic with scattered necroses on the upper leaves, an effect similar to that caused in these varieties by *Y* 4 and *Y* 5

TABLE 1. *The symptoms produced by four strains of virus* Y *in seven potato varieties*

Potato variety	Primary and secondary symptoms produced by strain			
	Y_1	Y_3	Y_5	Y_7
Majestic	Necrotic local lesions. Severe veinal necrosis and L.D.S.* of all except uppermost leaves, which show severe mosaic and crinkling	Some local lesions. Veinal necrosis and L.D.S. on lower and middle leaves. Upper leaves, veinal mosaic and rugosity	Necrosis and L.D.S. on few lowest leaves. Upper leaves, mild mosaic and slight deformity	No necrosis. Faint veinal mosaic
	Severe rugose mosaic, some necrotic spotting. Plants dwarfed, crinkled and mottled	Small, sprawling plant. Leaves pale, mottled and crinkled	Moderate, sprawling plants. Mild mosaic and rugosity	Fair plants, only slight reduction in size. Leaves pale and suspicious of faint mosaic
King Edward	Necrotic rings and spots and L.D.S. of middle and lower leaves. Severe mosaic and crinkling of upper leaves	Necrosis and L.D.S. of lower leaves. Upper leaves mottled and rugose	Veinal mosaic and rugosity. Slight necrosis of lowest leaves	Faint veinal mosaic. No necrosis
	Very small plants. Severe rugose mosaic	Small, sprawling plants. Bright veinal mosaic and crinkling	Sprawling, weak haulms. Veinal mosaic and ruffling of leaves	Fair plants. Leaves pale, little waved and faintly mottled
Doon Star	Necrotic local lesions and acute L.D.S.	Necrosis and L.D.S. of middle and lower leaves. Others crinkled	Veinal mosaic and deformity. No necrosis	Mild veinal mosaic
	Crippled plants. Severe rugose mosaic, with some necrotic spots	Small plants, severe mosaic and crinkling	Pale, mottled plants, sprawling, weak haulms	Good plants, slightly pale, but no obvious mosaic
Arran Banner	Necrosis and L.D.S. of middle and lower leaves. Upper leaves, severe rugose mosaic	Some necrosis and L.D.S. of lower leaves. Veinal mosaic and crinkling of upper leaves	Veinal mosaic and some crinkling. No necrosis	Faint veinal mosaic
	Dwarfed, sprawling plants. Leaves small, mottled and crinkled	Sprawling, poorly developed plants. Leaves mottled and waved	Moderate plants, but haulms weak and mottled	Good plants, but leaves paler and smaller than controls
Arran Pilot	Bright veinal mosaic, crinkling and waving	Definite mosaic. Some waving of leaves	Mild mosaic	Nothing definite
	Very dwarfed weak plants, maturing early. Leaves pale, small and mottled	Small plants, maturing early. Pale and sprawling	Weak sprawling plants, maturing early	Fair plants, but pale and maturing earlier than controls
Gladstone	Acute necrotic reaction. Local lesions and systemic necrosis soon cause death	Necrotic local lesions. Severe L.D.S. of all except uppermost leaves, which are brightly mottled	Necrosis and L.D.S. of middle and lower leaves. Upper leaves mottled and crinkled	Symptoms mainly mosaic and crinkling, but some necrosis of lowest leaves
	Minute plants, severely necrotic and dying when a few inches high	Small plants. Upper leaves mottled and crinkled, others necrotic and falling early	Small plants. Mosaic and crinkling of upper leaves, others L.D.S.	Fair plants. Some necrosis and L.D.S. of lowest leaves
Katahdin	Severe necrotic reaction. No mosaic symptoms. Local lesions and sometimes only few systemic lesions, but more often plant killed by generalized necrosis	Local necrotic lesions and generalized necrosis that usually kills rapidly	Severe necrotic spots and stripes on all except uppermost leaves, which are crinkled and mottled	Symptoms mainly mosaic and crinkling, but some necrosis of lower leaves
	Minute, acutely necrotic plants that soon die	Minute, acutely necrotic plants that soon die	Dwarfed plants with mottled crinkled leaves and some necrosis	Small plants with mottled, crinkled leaves and few necrotic spots

* L.D.S. = leaf-drop streak.

(Pl. 13, fig. 4). In tobacco the disease produced by virus *C* is relatively mild, comparable with that produced by *Y*6 and *Y*7.

The most striking differences in the symptoms resulting from the various strain-host combinations are in the production of necrosis in the first year of infection. Arran Pilot gives no necrotic symptoms with any of the strains, whereas Gladstone and Katahdin develop some necrosis with all, and the remaining varieties do with some strains but not with others. The results clearly indicate the difficulties of attempting to diagnose causative viruses from the symptoms shown by diseased plants in the field. All that can be done with any degree of certainty is to attribute leaf-drop streak to one or other strain of virus *Y*, but other diseases ranging from top-necrosis through severe mosaics to negligible mottles may also be caused by virus *Y*. The difficulty is not only that the same strain causes different symptoms in different varieties, but that with each variety there is an equally wide range in the type and severity of symptoms produced by different strains. In Majestic, for example, the differences between the top-necrosis caused by virus *C*, the acute leaf-drop streak caused by *Y*1, and the mild mosaic caused by *Y*7, are as great as the differences between the diseases caused by any one of these strains in different varieties.

Diagnosis from secondary symptoms is even more uncertain than from the primary, because leaf-drop streak, the one really characteristic symptom of virus *Y*, usually occurs in the first year only. The varieties Gladstone and Katahdin are exceptions, for they show considerable necrosis and leaf-drop in the second year of infection. Indeed, the reaction of these two varieties to most of the strains except *Y*7 is so severe that second-year plants usually succumb when a few inches high before they have formed any tubers. Sometimes, both under glass and in the open, such plants have given an exact reproduction of the first-year symptoms. At first the young shoots grow away vigorously and look normal, but when a few inches high they develop leaf-drop streak. We have tested such plants by inoculation to tobacco and have failed to detect virus *Y* in the shoots before symptoms were obvious. It seems that the virus must have become localized in the tubers and that some buds are able to grow normally at first, but later, perhaps because of the movement of food reserves from the tubers to the shoots, the virus enters and infects them. In the other varieties it is rare to see appreciable necrosis in the second year of infection, even when they are infected with strains that caused leaf-drop streak as a primary symptom. Secondary symptoms consist of mosaic, leaf deformities, dwarfing and early maturation. Occasionally, however, in Majestic and Up-to-Date we have seen the phenomenon of healthy shoots growing vigorously for some time and then suddenly developing the typical first-year symptoms of leaf-drop streak. This suggests that in these varieties tissues react necrotically only when they are well developed at the time of infection, and that mosaic symptoms are characteristic of tissues that are virus-infected soon after initiation. There is no appreciable difference between the virus content of leaves from plants in the first and second year of infection.

In general, the severity of symptoms in the second year reflects the severity of the primary symptoms; for example, with Majestic, the progeny of plants suffering from acute leaf-drop streak are small, deformed, brittle and mature very early, whereas the progeny of plants infected with *Y*6 and *Y*7 are only slightly dwarfed and mature only a little earlier than healthy plants. In spite of the relatively slight effects produced in Arran Pilot in the first year of infection, second-year plants may be much affected. They show no very distinctive leaf symptoms, but they are much reduced in size, mature early, and their cropping power is much reduced. Again, however, there is considerable variation from strain to strain, and *Y*1 has a much greater crippling effect than *Y*7. One fairly general and characteristic second-year symptom in plants infected with strains that allow the plants to reach a moderate size is that the stalks become prostrate much earlier than in healthy plants and sprawl untidily in all directions.

In addition to healthy plants, we have also inoculated strains of virus *Y* to plants already infected with virus *X*. In tobacco plants the two viruses together produce a disease of a different type and more severe than either does alone, but we have noticed no such synergistic effect in potato plants. The result of a dual infection seems simply to combine the separate effects of the two viruses, and the additional effect of virus *X* depends on the severity of the symptoms produced by the particular strain when present alone. Plants carrying virus *X*, or showing only faint mottles, reacted to virus *Y* in the same manner as healthy plants, and in no variety did the presence of virus *X* lead to the production of leaf-drop streak by strains of virus *Y* that did not cause this symptom when present alone.

Transmission by aphides

From the symptoms produced on the potato varieties used in this work, virus *C* is differentiated from the other strains of virus *Y* because it is the only one that caused top-necrosis. It was largely because of this property that virus *C* was originally described as a separate virus (Bawden, 1936), for it was not then realized what widely different diseases may be caused in one potato variety by different strains of one virus. Even this differentiation, however, is probably unreal and might well have disappeared had another range of varieties been chosen as differential hosts, for potatoes are known that react with top-necrosis to strains of virus *Y* that cause leaf-drop streak in such varieties as Majestic (Hutton & Bald, 1945; Hutton, 1946). Thus from symptomatology there would seem to be no reason for giving virus *C* any special status among the numerous strains of virus *Y*. There is, however, one feature of virus *C* which does make it anomalous and separates it sharply from the other strains of virus *Y* we have studied. This anomaly is our constant failure to get transmission of virus *C* by aphides that readily transmit the other strains.

The failure of *M. persicae*, the most efficient vector of virus *Y*, to transmit virus *C* has been reported by Cockerham (1943) and Bawden & Sheffield (1944).

The only experimental transmissions of virus *C* by aphides are reported by Bald & Norris (1945) from Australia. They obtained only two infections out of eighty-four plants colonized by over 1500 *M. persicae*, and do not appear to have tested these plants to ensure that the infections were caused by virus *C* and not some other strain of virus *Y*. Potato virus *C* occurs commonly in some old and little-grown varieties such as Edgecote Purple and Di Vernon, which react to infection with mosaic symptoms. In the field spread is rare, but Cockerham (1943) records three infections in potato seedlings growing in the vicinity of infected Edgecote Purple, and on two occasions we have found it causing top-necrosis in Majestic plants growing near to, but not in contact with, infected Di Vernon and Dargill Early. These results and observations suggest that occasional individual *M. persicae* or other potato aphis may be vectors although the bulk of such species are not, or that

TABLE 2. *Comparative transmission of potato viruses* C *and* Y *by different species of aphides*

Species of aphis	No. of aphides per plant	No. of infections	
		Potato virus *C*	Potato virus *Y*
**Myzus persicae* Sulz.	5–20	0/130	105/130
Macrosiphum solanifolii Ash.	10	0/10	5/5
Aphis (*Doralis*) *rhamni* Fons.	10	0/10	8/10
Aulacorthum circumflexum Buckt.	10	0/15	3/5
Aphis (*Doralis*) *fabae* Scop.	15	0/10	1/10
Aulacorthum solani Kalt.	10	0/10	1/10
Cavariella pastinacae L.	15	0/10	1/10
Macrosiphoniella sanborni Gill.	10	0/10	1/10
Myzus ornatus Laing	10–15	0/10	6/100
Hyperomyzus latysiphon Theob.	10	0/10	0/10
Nasonovia ribicola Kalt.	10	0/10	0/10
Myzus ascalonicus Doncaster	15	0/17	0/17

* Tests were made with *M. persicae* from different sources and to potato as well as tobacco. Tests with other species were all from tobacco to tobacco; in each test of a different species, control transmissions of virus *Y* were made with *M. persicae* to ensure that conditions were favourable. Using five aphides per plant, *M. persicae* regularly gave 100% transmissions.

some species may be a vector but is only a rare colonizer of potatoes. Our attempts to gain evidence for this hypothesis have failed. We have taken *M. persicae* from many different sources, from peach trees, Brassicae crops, and potatoes in different parts of Britain, but none has acted as a vector of virus *C*, though all transmitted *Y* 1. We have used many aphides per test plant, have attempted transmissions from potato to potato, potato to tobacco, and tobacco to tobacco, using various conditions of starvation before feeding on the source of infection, of length of infection feeding and of time on the test plants, but without success. We have also failed to transmit virus *C* by any other species of aphis, although as Table 2 shows many of these transmitted *Y* 1. The main feature of this table is the great range in the efficiency shown by the different species in transmitting virus *Y*. With *M. ornatus*, for example, using ten to fifteen individuals per test plant, usually less than one in ten

of the colonized plants became infected, whereas with *M. persicae* 100% transmission is usually obtained with two aphides per plant. This difference between the efficiency of different vector species would seem to provide a strong argument against the widely held view that viruses like potato *Y* are mechanically transmitted as contaminants on the aphides' mouthparts, but too little is known of the mechanism of transmission for any definite interpretation of the phenomenon. There is no reason to believe that infection can occur because of the cumulative effects of subminimal infection doses provided by several different insects. Indeed, all the evidence available suggests that infections are local and independent, and that the probability that a group of insects will cause infection is the probability that one or other of the members of the group would alone cause infection (Watson, 1936; Storey, 1938). Hence it seems likely that in such species as *M. ornatus* only a few individuals are capable of acting as vectors of virus *Y*. We have not attempted to study this by following the behaviour of individual aphides, but with maize-streak virus Storey (1932) found that some individual leaf-hoppers of the species *Cicadulina mbila* were vectors and others not, and that the ability to transmit is inherited in a Mendelian manner. Some such difference in the ability of individuals of aphis species to transmit virus *Y* provides the simplest explanation of our results, but there is as yet no conclusive evidence for this supposition.

All the strains we obtained from the field are readily transmitted to tobacco by *Myzus persicae*, but we have some evidence that they may differ from one another in the ease with which they are transmitted to potato. Table 3 shows the results

Table 3. *Comparative transmission of two strains of virus* Y *from tobacco to potato plants by* Myzus persicae

Virus strain	King Edward	Gladstone	Majestic	Katahdin	Total
*Y*1	*9/10 6/10	7/10 5/10	8/10 3/10	3/10 2/10	43/80
*Y*7	6/10 4/10	4/10 2/10	2/10 2/10	0/10 0/10	20/80
Total	25/40	18/40	15/40	5/40	63/160

* Numerator is the number of plants infected and denominator the number colonized. The results of two separate experiments are recorded. Katahdin plants were colonized with four aphides each, and the other varieties with two only.

of two tests in which *Y*1 and *Y*7 were transmitted from tobacco to King Edward, Gladstone, Majestic and Katahdin potatoes, using methods previously described (Bawden & Kassanis, 1946). Although there are great differences between the resistance of the different varieties, it will be seen that in each variety more infections were obtained with *Y*1 than with *Y*7, and that out of a total of eighty test plants used for each strain forty-three became infected with *Y*1 and only twenty with the less virulent *Y*7. This difference, which would give *Y*1 more opportunity for natural spread, may explain the fact that in the field strains of *Y* that give leaf-drop streak in Majestic seem to be more common than others. However, such strains are also the most easily detected, and in the absence of a survey there is no conclusive evidence on the relative prevalence of the different strains.

Discussion

Our results show that potato virus *Y* is far from being the single, uniform entity that it is widely assumed to be. Many different strains can be found in the field which differ from one another in the type and severity of symptoms they produce in potato plants. The range of diseases caused by strains of virus *Y* is as great as that caused by virus *X*, and, as the two ranges also overlap considerably, it is not always possible to tell from the appearance of a potato plant whether it is infected with a strain of *X* or *Y*. In discussing the relationships of viruses causing necrotic diseases of the potato, Bawden & Sheffield (1944) commented on the dangers in using symptomatology as a basis for grouping and classifying potato viruses, and the strain differences we have now demonstrated in virus *Y* serve only to emphasize these dangers. The fact that two viruses cause similar, or identical, symptoms in one variety is no reason to believe they are related. If they cause different symptoms, they are obviously not identical, but this fact alone provides no evidence as to whether the two are unrelated viruses or related strains. The production of a given set of symptoms is not an intrinsic property of either a virus or a host, but is the consequence of a host-parasite interaction, and a change in either host or parasite may alter the type of symptom produced.

During the course of our work the various strains of virus *Y* have remained constant, and we have no evidence that changes analogous to mutations occur with great frequency. However, the fact that the virus occurs in the field in strains of varying virulence shows that such changes do occur, for there can be little doubt that these strains have a common origin. It would seem that the most frequent form of mutant in virus *Y* is one identifiable by a change of virulence towards certain hosts, the form that has also been most frequently described in other viruses. The differences between virus *C* and the other strains of *Y*, however, go beyond this; they affect transmission by insect vectors and raise some interesting evolutionary problems. It is unlikely that virus *C* has originated recently as a mutant from an insect-transmitted strain of *Y*. A change in a virus leading to loss of transmission would have effects comparable with those of a 'lethal gene' in a higher organism, and it is difficult to see how such a mutant could get distributed in competition with readily transmitted strains. Virus *C*, however, does occur commonly in many old potato varieties and has been reported in locally grown types in Europe, South America and Australia. Thus it seems probable that in the past virus *C* was transmitted much more readily than it is to-day, and that its existence can now be attributed to the vegetative propagation of these old varieties, without which it might well be extinct. If this is so, a change in the insect vector rather than a change in the virus seems the most likely explanation of the present position. The manner in which such a change could come about is indicated by the results of transmission tests of virus *Y* with different aphides, which show that some species are occasionally vectors. If this is because occasional mutant individuals can transmit

whereas the bulk are inactive as vectors, a numerical change in the proportion of active to inactive individuals would determine whether or not a species is an important vector. The life cycle of aphides provides ample opportunity for such changes. During the summer months large populations develop, so providing abundant chances for mutation, while the catastrophic fall in numbers during the autumn and winter provides a mechanism for selecting mutants and drastically altering the proportions of different strains within the population. Unless the ability to transmit a virus is linked with some character of survival value to a species, such selections will occur purely by chance, and changes in either direction, i.e. to or from vector types, will be equally likely.

Other things being equal, the most prevalent strains of a virus will be those that are most easily transmitted. A mutation in a virus to a strain that is more easily transmitted immediately affords the mutant an opportunity of being selected by a vector and spread widely, free from competition with the parent strain. A mutation in the reverse direction, however, could have little chance of survival. Hence, if there has been no change in the ability of insects to transmit virus *C*, occasional spread by aphides, combined with vegetative propagation of the infected hosts, must account for its present distribution, and it would seem that the strains of virus *Y* now common have originated by mutation from *C* rather than the other way round.

A discussion of the evolution of virus diseases must from its nature be largely speculative and academic, but the problem has its practical side. Three things are concerned in determining the prevalence of an insect-transmitted virus, the virus itself, its insect vector and its hosts. None of these is constant, and a change in any one of them may affect the incidence of a disease. Even if virus *C* were as readily transmitted as the other strains of *Y*, it is still doubtful if it would be as prevalent as they are, for the most widely grown current varieties die when infected, so that the virus is eliminated from the stocks. Now that parents are known which are hypersensitive to other strains of virus *Y* (Hutton & Bald, 1945), attempts are being made to produce commercial varieties with this property. The value of hypersensitive varieties in controlling virus *X* is widely recognized, but virus *X* is not insect-transmitted, and strains that do not cause top-necrosis have little opportunity of entering stocks of such varieties. The position is different with virus *Y*, which can be carried considerable distances by aphides. In breeding for hypersensitivity to virus *Y*, tests should be made with the largest possible number of virus strains, for some may not cause lethal diseases and there is little doubt that these would soon be brought into such varieties and spread in them by aphides. Our transmission tests to different varieties suggest an additional factor that might be equally worth breeding for, namely, resistance to infection. The variety Katahdin, for example, is much more difficult to infect than any other we have studied. It also reacts severely when infected, with some strains almost to the extent of being hypersensitive and with others sufficiently for second-year plants to leave few or no tubers to

perpetuate the strains in the stock. The combination of resistance to infection with intolerance when infected would seem well worth seeking, though potato viruses are so variable that it is uncertain for how long a variety bred for any specific feature will retain it when cultivated over a wide area.

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EXPLANATION OF PLATE 13

Fig. 1. Majestic potato plants of the same age photographed 6 weeks after infection with *Y* 1 (right-hand) and *Y* 7, showing severe leaf-drop and dwarfing caused by former and the negligible effects of the latter.

Fig. 2. Comparable leaves from King Edward plants in the second year of infection with *Y* 1 (right-hand) and *Y* 7, showing the brighter mosaic and greater dwarfing caused by the former.

Fig. 3. Katahdin plants showing severe necrosis caused by *Y* 1 (right-hand) and mild effect of *Y* 7. Photographed 1 month after infection.

Fig. 4. Plants of the variety Gladstone photographed 5 weeks after infection with potato virus *C* (left-hand) and *Y* 5. Both strains cause similar symptoms in this variety, dropping of lower leaves and crinkling of upper.

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BAWDEN and KASSANIS—*Behaviour of some naturally occurring strains of potato virus* Y

THE 'PHLOEM NECROSIS' VIRUS DISEASE OF TEA IN CEYLON

III. FURTHER CHARACTERIZATION OF NECROSIS IN THE LEAF

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(With Plate 14 and 7 Text-figures)

Phloem necrosis due to the virus disease of that name in tea is fully described as it affects the leaf, on which diagnosis is chiefly based. Originating in the protophloem, it may extend inwards to the metaphloem and outwards to the pericycle, causing the breakdown and discoloration of the cells and their eventual death and obliteration, cell enlargement, and the production of new thin-walled cells by hyperplasia. The condition is termed 'true necrosis' to distinguish it from the non-pathogenic 'false necrosis', of unknown cause, which originates typically in the metaphloem but may have the same histological effects except for the absence of hyperplasia. In the petiole, the visual distinction between 'true' and 'false' necrosis on the basis of their position as seen in a transverse section of the bundle is relatively easy, and the continued use of this method of diagnosis is recommended. No such distinction can reliably be made in the midrib, where 'false' necrosis often occurs in the same position as 'true' necrosis, i.e. immediately within the pericycle. This is interpreted ontogenetically in terms of the smaller total width of the phloem in the midrib as compared with the petiole bundle; it effectively prevents the use of the midrib for diagnosis. The observations are discussed in terms of the inherent properties of the phloem, as affected by viruses and other agencies reported to have caused necrosis, among plants in general.

No detailed account of the histology of phloem necrosis caused by the virus disease of that name in tea has yet been given. To conclude the writer's study of this disease (Bond, 1944*a*, *b*), such an account is now presented for the petiole and midrib of the leaf, for which alone there is adequate material available. The precise characterization of phloem necrosis as it affects these organs has been undertaken in order to provide a more accurate basis for diagnosis (particularly to establish the distinction from the non-pathological 'false necrosis', already briefly described), and to permit a more adequate comparison of the disease in tea with the many other phloem-necrotic diseases to which it may, possibly, be related.

Material

The account is based on about 200 slides of freehand sections made by the writer in 1940–3, forwarded by courtesy of the Director of the Tea Research Institute. The sections were cut as part of the routine examination of experimental plants including older bushes, known to be necrotic, and many young seedlings and cuttings under inoculation by various methods. The material, either whole leaves or leaf-tips, was

usually placed in 50% alcohol on collection; the sections were mounted in Canada balsam after rapid staining in light green in clove oil, just sufficient to bring out the contrast between the necrotic and other cell walls.

In this paper, the virus necrosis will be referred to subsequently as 'true necrosis', in order to distinguish it from 'false necrosis'. The term 'phloem necrosis' will be applied to the disease as such, rather than to its histological effects on the phloem.

True necrosis

Tissues involved. Bond (1944*a*) stated that in transverse sections of the petiole or midrib bundle, the necrosis should be 'peripheral in origin, lying on the outer edge of the phloem and close within the pericycle'. Further experience has shown that this is only approximately correct, and that in moderately to severely necrotic leaves the pericycle itself is affected, the necrosis also spreading inwards for a considerable distance towards the cambium. The point is illustrated by Text-fig. 1 A–E (left-hand figures), showing the disposition of necrotic tissue in the petiole bundles from mature leaves, variously affected. As described more fully in a subsequent paragraph, the pericycle when necrotic may become virtually obliterated, or it may be subject to an increase in width by hyperplasia. These changes occur both in the petiole and in the midrib; occasionally they are found side by side in the same section.

Histological effects. Even in severely diseased shoots, where the young leaves are curled as soon as they unfold from the bud, necrosis is not usually evident until growth has more or less ceased, i.e. until the primary vascular structure has attained almost its full development, as previously described by Bond (1942). At this stage, the protophloem is in an advanced state of obliteration, many of its cells having become unrecognizable except as local thickenings in the walls of adjacent cells. It is here that necrosis usually first appears; it resembles in its effects an acceleration of the normal process of obliteration, with the difference that the crushed cells and their thickened walls become discoloured owing to the accumulation of a yellowish brown, amorphous substance resembling wound gum. The discoloured walls give a slight reaction with phloroglucinol, not increased by heating. Difficulties in diagnosis arise from the fact that a similar discoloration of the obliterated protophloem can occur, occasionally, in association with 'false necrosis' affecting otherwise healthy bushes.

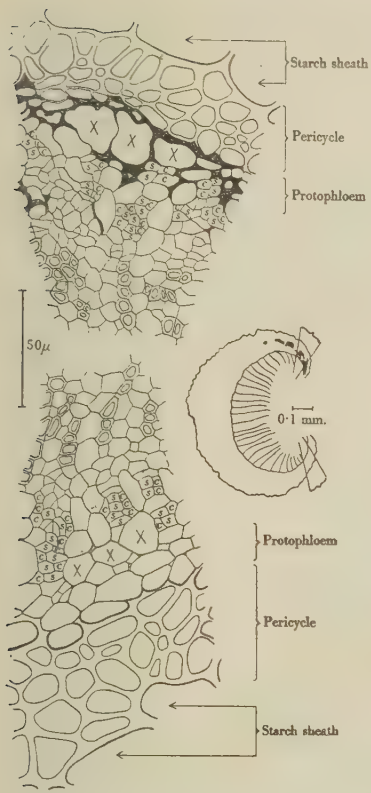
As necrosis proceeds, more and more cells become obliterated, whilst others exhibit a compensatory tendency to hypertrophy (cf. Sheffield, 1943). This was for a long time overlooked, owing to the normal diversity in size of the cells principally affected, either of the phloem parenchyma or of the pericycle. In any given section it can best be demonstrated by a careful comparison of equivalent necrotic and healthy sectors, with reference particularly to the groups of parenchyma cells associated with the ending of the several medullary rays (Text-fig. 2; Pl. 14, fig. 1). This phase of 'primary hypertrophy' (Esau, 1935) is of variable duration, depending

Text-fig. 1 A–E. Occurrence of *true* (left) and *false* (right) necrosis in the phloem of the petiole bundle in transverse sections, at increasing levels of severity. Necrotic tissue is shaded solid. The bundles are shown in outline with the phloem, bounded by the *outer* edge of the pericycle, uppermost. The drawings were made with the aid of a projection apparatus, at an original magnification of × 100.

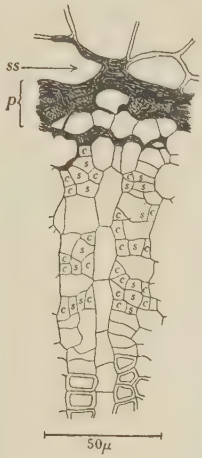
on the circumstances. Thus, where the total incidence of necrosis is low (as in Text-fig. 1 A–B), its effects on the individual cells are relatively slight; hypertrophy is not usually pronounced, and the enlarged cells appear able to persist, as such, for a long time. The inference here is that the onset of necrosis has been relatively delayed. At an intermediate level (Text-fig. 3) the crushing effect appears to predominate so that the cells undergoing hypertrophy are soon obliterated, their remains accumulating as a dense necrotic residue in which indistinct cell cavities may for a short time remain discernible. The pericycle is now increasingly affected, and may locally be destroyed. Finally, in the most heavily necrotic bundles (Text-fig. 4; Pl. 14, figs. 2–5), i.e. where the onset of necrosis may be presumed to have been at its earliest, there occurs a further, hyperplastic response whereby the 'giant cells' (as they may now be termed) become subdivided to form pockets of thin-walled, variously arranged cells which are themselves subject to further division. It is this hyperplastic response which, alone, *absolutely* distinguishes 'true' from 'false' necrosis. It originates within or just inside the pericycle, and, in the midrib, has the effect of preventing the normal lignification of that tissue. Very occasionally it extends beyond the pericycle to a few cells of the adjoining starch sheath. The hyperplastic tissue, being colourless, by its continued division tends to become surrounded by an accumulation of necrotic, discoloured residues. Consequently, there is in this final stage of the disorder not only an increase in the total width of the region affected, but also a tendency for the necrosis itself to become discontinuous in its distribution or to be separated into distinct inner and outer zones (Pl. 14, fig. 4). In the latter event, i.e. where the hyperplastic divisions are predominantly radial, there is a suggestive similarity in effect to that which is produced normally, in the stem, by the early activity of the phellogen (Pl. 14, fig. 5).

False necrosis

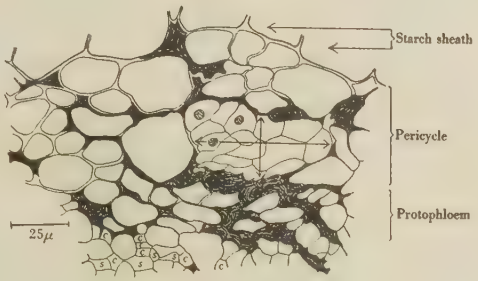
Occurrence. The term is applied (Bond, 1943) to a non-pathological necrosis of the phloem in the leaves of plants of various ages, which was believed originally to be induced by injury or other unfavourable conditions affecting the roots. Thus, the necrosis is most prevalent in young seedlings raised in peat and, on one occasion, was associated with an abnormal failure of elongation of the tap-root; no result, however, emerged from an experiment in which the roots were deliberately mutilated or cut short. On transplanting a batch of affected seedlings into soil, in the field, the incidence of necrosis in them gradually diminishes, though in some plants the condition appears able to persist indefinitely. It is not associated with leaf-curl or any other external symptom, nor can the likelihood of its occurrence be predicted from the varietal type. Hochapfel's (1940) account of a similar though much more severe necrosis in the phloem of tomato roots suggests for future investigation the possible effect of exposure to cold, i.e. to temperatures barely in excess of those causing evident frost injury.



Text-fig. 2



Text-fig. 3



Text-fig. 4

Text-fig. 2. *True* necrosis; comparable necrotic (above) and healthy (below) sectors of the petiole bundle in transverse section, as in the diagram inset. The protophloem is principally affected. The three cells marked, in the centre of that tissue, are hypertrophied, as may be seen by comparing them with the corresponding cells of the 'healthy' sector (cf. Pl. 14, fig. 1). In this and subsequent text-figures the necrosis is indicated by shading, and the sieve tubes and their companion cells are labelled *s* and *c* respectively. The figures are from camera-lucida drawings made with a no. 2 eyepiece and $\frac{1}{12}$ in. oil immersion objective, at an original magnification of × 1000.

Text-fig. 3. *True* necrosis; median sector of petiole bundle in transverse section, affected to about the same degree as the left-hand section of Text-fig. 1C. The pericycle (*p*) is virtually obliterated, with only a few cell cavities remaining. The necrosis has extended outwards to the adjacent starch sheath (*ss*) and inwards to the protophloem, in which there is a moderate degree of hypertrophy.

Text-fig. 4. *True* necrosis; details of advanced necrosis with hyperplasia in the petiole bundle in transverse section. A number of enlarged pericycle cells is shown, one of which has given rise to a pocket of thin-walled hyperplastic tissue (extending to the limits of the arrows). The thin-walled cells have prominent nuclei, three of which appear in the section (cf. Pl. 14, fig. 3).

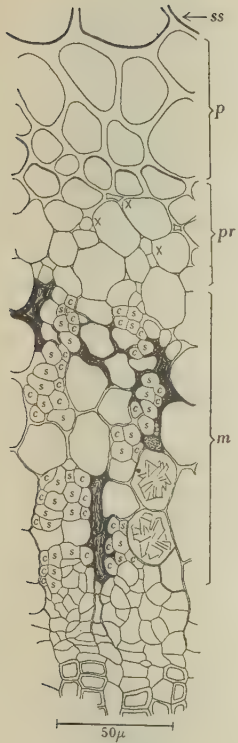
Characteristics. False necrosis was previously described (Bond, 1944*a*) as 'affecting the inner region of the phloem and with a marked tendency to radial development in association with the "medullary" rays'. For the petiole, as shown in Text-fig. 1 A–E (right-hand figures), this may be allowed to stand; from which it follows that the gross distinction between 'false' and 'true' necrosis is here comparatively easy (cf. Text-fig. 5). For the midrib, however, no such distinction can be made, it being now realized that false necrosis affects here the outer edge of the phloem at least as often as it does the inner regions of that tissue (Text-fig. 6). The apparent contradiction may be resolved by considering the position of false necrosis in relation not to the pericycle or other regions of the periphery but to the cambium in the centre. The necrosis occurs, in fact, at a fairly constant distance from the cambium, equal to the width of one or two layers of phloem elements. The distribution suggests the comparatively late onset of necrosis and its restriction, subsequently, to tissues at a given stage of development; probably those in which the sieve tubes have reached 'maturity' according to Esau's (1939) definition as accepted, for tea, by Bond (1942). Thus, the apparent convergence of the necrotic zone towards the pericycle, in the midrib as compared with the petiole, is the result merely of the smaller total width of the phloem in the former; this may be seen also in the latter by comparing the narrow lateral extremities of the bundle with its more representative median parts (Text-fig. 7). It should be noted, however, that although the obliterated protophloem becomes heavily necrotic under these circumstances, the pericycle itself is scarcely affected.

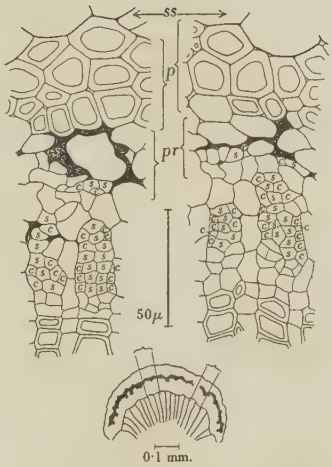
The chief histological effect of false necrosis is a thickening and yellowish brown discoloration of the cell walls, principally of the ray parenchyma and adjacent phloem elements, as shown in Text-fig. 5. Occasionally, the cell cavities may become blocked. As with true necrosis, the discoloured tissues react slightly with phloroglucinol. Where, as explained above, the necrosis extends to the protophloem, there are frequent indications of cell enlargement, *but never any hyperplasia* (Text-fig. 6).

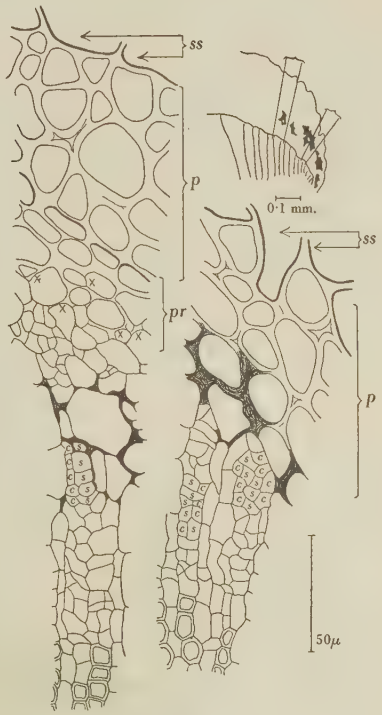
Conclusions

The problem of diagnosis. That the phloem necrosis disease of tea presents exceptional difficulties in its early diagnosis has already been established. They result not only from the need for distinguishing between true and false necrosis, but also from the lack of correlation between the internal, histological effects of the disease and its external manifestations. Reasons have been given for the need for basing the diagnosis of the disease principally on the leaf, which, in practice, meant the examination of the petiole where there was ample foliage available, and of the midrib where (as in young seedlings) a whole leaf could not be spared.

Petiole examination is used in the field where, according to the elevation, *jât*, and other circumstances, between a quarter and a half of the records may be diagnosed by this method alone, i.e. in the absence of external symptoms. Its use







Text-fig. 5 Text-fig. 6 Text-fig. 7

Text-fig. 5. *False* necrosis; median sector of petiole bundle in transverse section, roughly corresponding to the right-hand section of Text-fig. 1 D. Necrosis is confined to the metaphloem (*m*), the starch sheath (*ss*), pericycle (*p*) and protophloem (*pr*) being unaffected. Groups of normal obliterated protophloem elements (i.e. with the cell walls thickened but not discoloured) are indicated by the crosses.

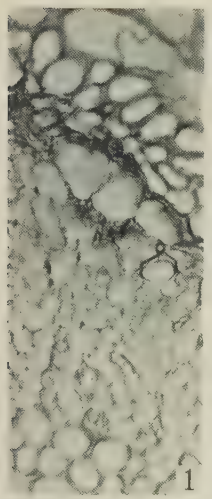
Text-fig. 6. *False* necrosis; median sectors of midrib bundle in transverse section, as in the diagram inset. Necrosis affects principally the protophloem (*pr*) and in consequence lies close within the (lignified) pericycle (*p*). There is locally some evidence of hypertrophy, as in the left-hand figure. The symptoms here would not be distinguishable from *true* necrosis.

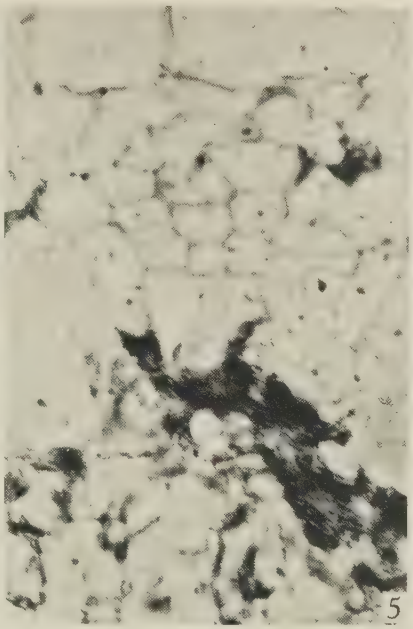
Text-fig. 7. *False* necrosis; sectors of petiole bundle in transverse section, as in the diagram inset. The more median (left-hand) sector is comparable with that shown in Text-fig. 5, i.e. with the necrosis, in the metaphloem, clearly separated from the pericycle (*p*) by the protophloem (*pr*), in which occur groups of normally obliterated elements (as indicated by the crosses). The lateral (right-hand) sector, in which the total width of the phloem is only about two-thirds as great, shows the necrosis lying immediately within the pericycle.

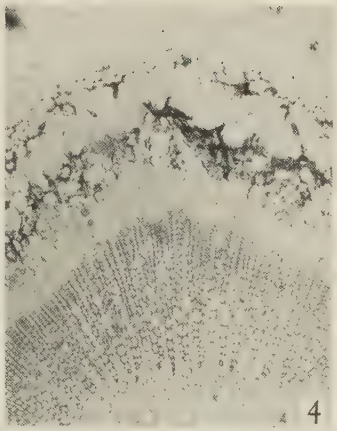
is vindicated by the examples illustrated in Text-fig. 1A–E, and by the further observation that students and others in this Department (persons unacquainted with true and false necrosis except through the bare definitions quoted above, from the first paper of this series), being given an assortment of similar diagrams, were able on the average to place twenty-three out of twenty-eight, or more than 80%, in their correct categories. An investigator with a few months' first-hand experience of the disease might be expected to attain an accuracy approaching 100% in such a test. It may be argued that the visual distinction between the two types of necrosis becomes impossible, in the petiole, when both occur simultaneously, or when either is confined to the lateral extremity of the bundle. This is conceded; but, in the field, such a difficulty can usually be avoided by examining other leaves from the same bush.

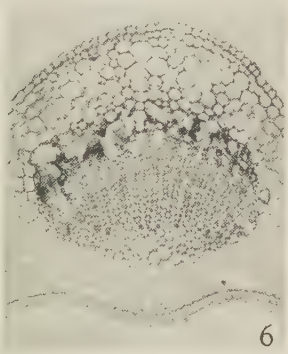
For the midrib, as already explained, the position of necrosis, whether in the middle of the phloem or at the periphery, is no longer diagnostic. All that can be said in this connexion is that, so far, only true necrosis has been observed to extend into the pericycle. With this exception the distinction between true and false necrosis has here to be attempted on histological characteristics which, in the petiole, could safely be ignored for the purposes of diagnosis. Since both types of necrosis react slightly with phloroglucinol (cf. Sheffield, 1943), the distinction can be made, with certainty, only in those instances where true necrosis has been succeeded by hyperplasia; and, in fact, it is at this phase that the pericycle is chiefly involved (Pl. 14, figs. 4–6). For the early recognition of the disease, sectioning the midrib has proved both unreliable and misleading.

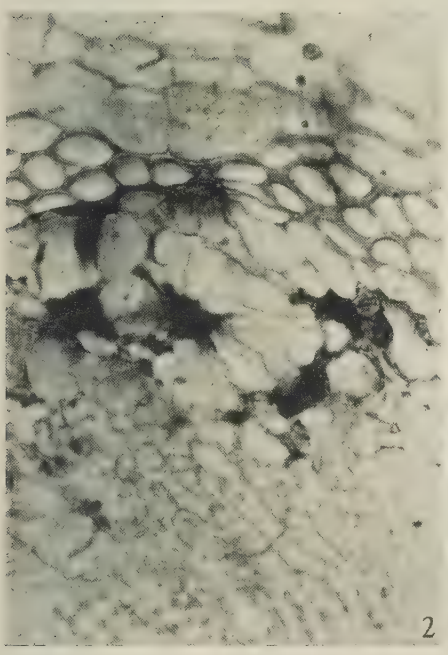
Fundamental considerations. Esau (1938*a*) concludes that 'the phloem is, in general, rather easily injured and responds with degeneration to different stimuli...', i.e. both of virus origin and otherwise. This is certainly true for tea, perhaps to an unusual degree. In tea phloem, the degenerative changes so far observed include necrosis proper, i.e. the breakdown and discoloration of cells leading to their eventual death, cell enlargement, and abnormal multiplication of the enlarged cells by hyperplasia. The first two are quite generalized responses of which the second alone is restricted to a particular region, namely, the protophloem and adjacent parenchyma (including the pericycle, when this is not lignified). Bond (1942) has already noted the 'general increase in size of the parenchyma cells adjacent to the sieve-tubes' that occurs as a normal concomitant of protophloem obliteration in tea; whilst Esau (1938*b*) quotes numerous examples of the further enlargement of cells in this region, destined to be recognized later as constituting the pericycle. Thus, it may be that a tendency to hypertrophy is inherent in the protophloem parenchyma and that the realization of this tendency follows as a consequence of necrosis, however caused. The further response, of hyperplasia, is apparently specific to the virus, being absent from false necrosis; but this, too, may be derived from an inherent tendency of the tissues affected, as witnessed by the pericyclic origin of the phellogen in tea. Artschwager's (1937) observation that phellogen activity in sugar-

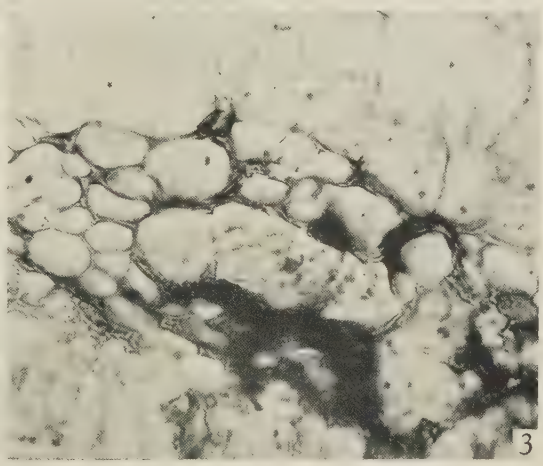












BOND—*The 'phloem necrosis' virus disease of tea in Ceylon*

beet rootlets was stimulated by the occurrence in adjacent sectors of phloem necrosis (i.e. a 'false necrosis'), due to unfavourable soil conditions, is of interest in this connexion. Whatever its real significance, the occurrence of hyperplasia in association with the phloem necrosis virus disease of tea serves to relate the latter more clearly with others such as the similarly named disease of American elm (McLean, 1944), and curly-top of sugar beet (Esau, 1935). As already indicated by Schneider (1945), there may also be a relationship with the buckskin disease of peach and cherry; in which disease, too, there has been the same need to distinguish the histological effects of the virus from those of other, non-pathogenic agencies.

I am indebted to the Director of the Tea Research Institute of Ceylon, and to Dr C. H. Gadd, for placing the slides and other data at my disposal; and to Mr E. Barron of Sheffield University for taking the photographs.

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EXPLANATION OF PLATE 14

All are examples of *true* necrosis, in transverse sections; figs. 1–5 from the petiole, and fig. 6 from the midrib.

Fig. 1. Part of Text-fig. 2, showing moderate degree of hypertrophy, without hyperplasia. (× 380.)

Fig. 2. Severe necrosis in the protophloem, with local obliteration of the pericycle. In the centre of the section is a group of thin-walled cells, radiating outwards from the dark necrotic mass on the left; they are almost certainly hyperplastic in origin. (× 380.)

Fig. 3. Same as Text-fig. 4. (× 380.)

Fig. 4. Advanced necrosis, with hyperplasia. Originating immediately within the pericycle, the hyperplastic tissue has divided the necrosis into well-defined inner and outer zones. (× 80.)

Fig. 5. Detail of the hyperplastic tissue, from the centre of the previous figure. (× 380.)

Fig. 6. Advanced necrosis in the midrib bundle. The pericycle is severely affected, its normal lignification having been almost entirely prevented. (× 80.)

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THE DEVELOPMENT OF INTERNAL BOLL DISEASE OF COTTON IN RELATION TO TIME OF INFECTION

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(With Plate 15 and 2 Text-figures)

Inoculations of *Nematospora* spp. into cotton bolls at successive weekly intervals from flowering to maturity show that the symptoms produced are closely dependent upon the stage of development reached by the boll when infected. Details are given of the nature and degree of the staining and of the effects upon weights of seed and lint finally produced. During the first 4 weeks of life bolls are in the stage of rapid growth and differentiation, and infection is followed by severe disorganization of all boll structures and complete commercial loss. Bolls infected later in life develop typical stained lint, but without general breakdown of other tissues, and the degree of staining diminishes steadily with the age when infected, until, towards maturity, little or no effect is produced.

Subsidiary experiments show that inoculations of sterile water can cause death of the seed embryos, presumably by plasmolysis, in bolls up to 3 weeks of age. The effects upon the seed and consequently upon the lint following death of the embryo caused by such treatment are shown to be similar to those following feeding by uninfected insects of the genus *Dysdercus*, which are the normal vectors of internal boll disease.

Staining of the lint by *Nematospora* is due to the post-mortem discoloration of the protoplasm of the lint hair, and its extent consequently varies inversely with the degree of vacuolation, which increases with maturity. The mode of action of the fungus and the evidence suggesting that a toxin is involved are discussed.

The bearing of the age-damage relationship on the losses due to internal boll disease in African cotton-growing countries is considered, and the advantages of promoting a steep flowering curve are emphasized.

I. Introduction

Internal boll disease, caused by *Nematospora* (*Ashbya*) *gossypii* Ashby & Nowell, *N. coryli* Peglion and related species, and transmitted chiefly by species of *Dysdercus* (Pyrrhocoridae), is one of the most serious and widely distributed afflictions of cotton in tropical and subtropical regions. In an earlier paper describing investigations at the Cotton Experiment Station, Barberton, South Africa in 1933, it was noted that the characteristic symptoms of internal boll disease were less pronounced in bolls which were 6 weeks old when inoculated with *Nematospora* than in those inoculated at 4 weeks of age (Pearson, 1934). Experiments were therefore undertaken in 1934 to determine more precisely the relation between the age of cotton bolls at the time of their infection and the nature of the damage resulting. The results have been briefly noted (Pearson, 1935), but pressure of events has hitherto prevented any fuller description of the work. Further observations in 1934, 1935 and 1938 on the development of the effects of inoculations with sterile water, spore suspensions and toxic extracts of *N. gossypii* are also recorded.

II. The relation of the degree of damage caused by *Nematospora* to the age of boll at the time of infection

Methods

Flowers of the African Upland variety U4/920 were bagged, to exclude insects, on 21 March 1934, and at subsequent weekly intervals a sample of forty normal bolls from this material was inoculated by syringe with 0·2 c.c. of a standard spore suspension in every lock, the boll surface being sterilized with methylated spirits before and after inoculation and the puncture sealed with 'Durofix'. To prevent the needle-point becoming plugged with tissue a preliminary puncture was made in the boll wall with a sterile solid needle. Subsequently a hypodermic needle was used having the end sealed with solder and a minute hole in the side just behind the tip.

Standard spore suspensions were obtained each week by shaking up with 15 c.c. sterile water a loopful of mycelial material collected from the centre of a 1-week-old colony of *Nematospora* grown on potato-dextrose-agar at constant temperature. The original isolations from naturally infected bolls consisted of two species, *N. gossypii* Ashby & Nowell, and what appeared to be *N. coryli* Peglion. Later isolations of apparently identical strains were identified at the Imperial Institute of Mycology by the late Mr S. F. Ashby as *N. gossypii* Ashby & Nowell and *N. phaseoli* Wingard.

Each week eighteen bolls were inoculated with *N. gossypii* and eighteen with *N. phaseoli*, whilst four were inoculated with 0·2 c.c. sterile distilled water in each lock as controls. In this and subsequent experiments here described the bolls were not removed from the plants, being rebagged after inoculation to continue their development.

Those shed were collected immediately, the rest, after they had split. After drying in a warm-air cupboard the whole boll and then each lock of seed cotton was weighed separately, thus giving the weight of dry carpels by subtraction. Each lock was graded for staining on an arbitrary scale. Ginning percentages were determined on the bulked material.

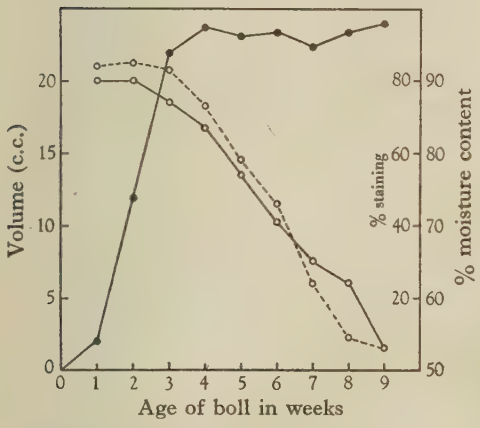
Some material was damaged by bollworms or by direct infections of *Xanthomonas* (*Bacterium*) *malvacearum* E.F.S. and had to be abandoned. In a few cases secondary contaminations were detected, but these were rarely serious, and, as the controls appeared equally liable to them, such material was included for measurement.

Boll diameters, volumes and moisture contents were determined on parallel random samples of 30–35 bagged bolls each week, the fresh weight and boll volume being determined by weighing in air and in methylated spirits. The seed cotton (i.e. seeds with developing lint attached) was then removed and the carpels (i.e. capsule walls, interlocular septa, central columella and calyx) weighed, the

fresh weight of seed cotton being obtained by subtraction. Both were then oven-dried to constant weight in paper envelopes, cooled in a desiccator and reweighed rapidly, using Joly spring balances.

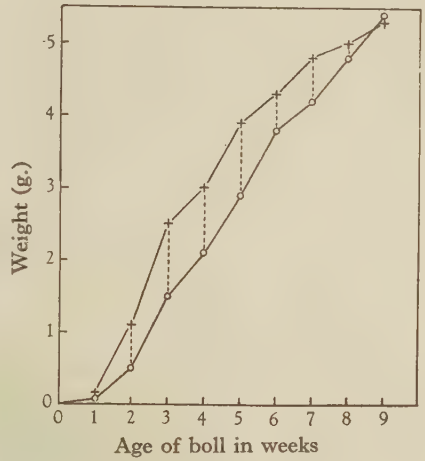
Results

(*a*) *Normal growth of the boll.* The boll-development studies confirmed the observation made on Egyptian cotton by Balls (1915), that growth of the boll envelopes is extremely rapid early in life but ceases between weeks 3 and 4.



Volume of boll ●——●
Moisture content of seed cotton O——O
Degree of staining O - - - O

Text-fig. 1. Relation of boll age to boll volume, moisture content of seed cotton and intensity of staining.



Dry weight of seed cotton per boll at time of inoculation O——O
Dry weight of seed cotton per boll at maturity from bolls inoculated at different ages +——+
The length of the broken vertical lines represents the additional growth achieved after inoculation.

Text-fig. 2. Relation of boll age to dry weight of seed cotton at time of inoculation and at maturity.

Changes in boll volume are shown in Text-fig. 1. Boll diameters averaged 1·50, 2·75, 3·30 and 3·35 cm., and dry weight of carpels 0·21, 0·80, 1·33 and 1·80 g. at the end of the first 4 successive weeks, after which no significant changes occurred. Only damage inflicted in the first 4 weeks of life can therefore affect the boll's external dimensions.

Dry weight of seed cotton, by contrast, increased steadily throughout boll life, as shown in Text-fig. 2; its moisture content fell from 90% in the first 2 weeks to about 50% at 9 weeks (Text-fig. 1), while over the same period that of the carpels

TABLE 1. *Summary of results of inoculations with* Nematospora *spp. and sterile water*

Group	Age of boll in weeks at inoculation	Inoculum	Shedding effect	Opening of bolls not shed	Boll growth	Staining	Lint development	Effect on seeds	Occurrence of *Nematospora*
I	1 (Pl. 15, fig. 3)	*N. gossypii*	Majority shed after 1–2 weeks	Aborted; slightly split basally but not apically	Ceased after inoculation	Dark brown, general	Lint broken down, locks mummified or resembling walnut kernel	Dead	Difficult to find in shed bolls; spores copious in rest
		N. phaseoli	As above, but slightly later	Aborted, though split apically and carpels partly reflexed	As above but slightly later	Do.	Do.	Do.	Sporangia readily found in shed bolls; spores copious in rest
		Sterile water	Nil	Split; carpels fairly fully reflexed	Small	Brown speck at point of inoculations	Locks 'waisted' due to very immature lint at point of inoculation; elsewhere poor but clean	Majority dead	Some traces of bacteria
II	2 (Pl. 15, fig. 4)	*N. gossypii*	One-third shed	Premature; split equatorially but not apically	Small	Dark brown, patchy	Locks hard, lint reduced to papery membrane closely enfolding seeds	Poorly developed	Spores present, not numerous
		N. phaseoli	Nil	Split apically; carpels partly reflexed, much contorted	Do.	Do.	Lint partly as above, elsewhere very immature, matted	Do.	Spores numerous
		Sterile water	Nil	Fairly good, carpels not fully reflexed	Much larger than above	Brown speck at point of inoculation	Locks strongly 'waisted' at point of inoculation	Dead at point of inoculation	No trace
III	3	*N. gossypii*	Nil	Split apically; carpels partly reflexed, contorted	Reduced	Severe round point of inoculation but also diffused throughout lock and up suture line	Locks hard; lint membranous centrally, fluffier apically, strongly webbed	Some poorly developed	Spores present
		N. phaseoli	Nil	Do.	Do.	As above but less stained at extremities	As above, but locks larger	Do.	Do.
		Sterile water	Nil	Normal	Normal	Brown speck at point of inoculation	Small area of immature lint where inoculated; not 'waisted'	As above at point of inoculation	No trace
IV	4 (Pl. 15, fig. 5)	*N. gossypii* and *N. phaseoli**	Nil	Not fully reflexed but better than in week 3	Reduced	Pronounced in small area round point of inoculation, on suture lines and inner lock faces	Central mass of immature fibres, fluffier externally; very pronounced webbing	Apparently normal	Spores present
		Sterile water†	Nil	Normal	Normal	Nil	Slight trace of immaturity at point of inoculation	Normal	No trace
V	5	*N. gossypii* and *N. phaseoli*	Nil	Fairly well opened	Slightly reduced	Diffused generally throughout lock	Locks still hard but less compact and less webbing than in week 4	Apparently normal	Spores present
VI	6	Do.	Nil	Do.	Distinctly larger than above	General internally, confined to suture line externally	Locks springy, little external webbing	Do.	Do.
VII	7	Do.	Nil	Good, though still poorer than controls	Normal	Diffuse streak on suture line, sometimes general internally	Generally mature, little webbing, no matting	Do.	Traces only
VIII and IX	8–9	Do.	Nil	Good, indistinguishable from controls	Do.	Slight traces on suture line	Slight traces of immaturity at point of inoculation	Do.	Do.
X	—	Not inoculated	Nil	Normal	Do.	Nil	Silvery, silk-like, traces of immaturity	Do.	Nil

* In this and subsequent inoculations, no difference in effect of species.

† All subsequent inoculations with sterile water similar.

fell from 83 to 77%; both dropped sharply at boll splitting, at between 9 and 10 weeks.

These results have since been generally confirmed by more precise experiments at Barberton (Rainey, 1940).

(*b*) *Effect of inoculation upon boll structure and appearance.* In Table 1 are set out in compact form the effects of the inoculations. Some bolls treated early in life shed or aborted soon afterwards; others, seriously affected, split prematurely, but the great majority opened at the usual time, i.e. some 10 weeks after flowering. The notes refer to the final state of the bolls. Typical examples of each treatment are shown in Pl. 15, fig. 1.

To sum up the results: these inoculations have produced symptoms identical with and covering the entire range of symptoms found in cotton crops following the attacks of insect vectors of *Nematospora*. They show clearly that the extent of the damage caused by *Nematospora* is inversely correlated with the age of the boll at the time of infection.

Growth of bolls inoculated at 1 and 2 weeks of age is almost completely arrested and the bolls either shed or abort, the seeds are killed and the lint is frequently reduced to a darkly stained papery membrane. Some of the effects observed were partly due to the water inoculated with the fungus, which can itself cause death of the central seeds of the lock, with resultant breakdown or immaturity of the lint, reduction in boll size and premature opening.

The size of bolls 3 and 4 weeks old when inoculated is still affected but much less severely; lint is heavily stained but not completely broken down, and the carpels open, but are contorted and only partially reflexed; the centre of the lock is 'hard', the lint not expanding to separate all the seeds; the most characteristic feature is 'webbing', due to the adhesion of the edges of the split carpels to strands of lint which, as the former open, are stretched tightly across from those to the parent seeds. Sterile-water inoculations had no effect upon these older bolls, apart from the production of small areas of immature lint in bolls inoculated when 3 weeks old, and the whole of the symptoms seen can be ascribed to the effects of *Nematospora*.

Inoculations at 5, 6 and 7 weeks of age show little or no effect on boll size and opening; lint is not broken down and shows little or no 'webbing', but it is diffusely stained, the extent and depth of the colour, from dark brown to straw yellow, decreasing with the age at which inoculations are made. It is this type of boll which produces the stained cotton of commerce.

Bolls inoculated at 8 and 9 weeks of age show no effects other than traces of discoloration and immaturity.

The method of inoculation used has two drawbacks, the first being the dosage of fungus and the second the effect of the water in which it is conveyed. The dosages used were presumably much in excess of any which could be introduced by an insect vector, but there was no suggestion that they were swamping the effects of boll age, which quite clearly was the dominating factor. The effects of the water

undoubtedly obscured those of the fungus in very young bolls, but not in those 3 or more weeks old, and do not impair the main argument. These experiments have been repeated recently at Barberton (Wickens, 1947) using very much smaller quantities of inoculum, with results which appear to agree very closely with those above.

(*c*) *Effect of inoculation upon weight of seed cotton.* The effect of *Nematospora* in reducing the commercial yield of cotton is twofold. First, bolls or locks which are severely affected are not picked at all; the whole of groups I–III and a good many of group IV would therefore be a dead loss. Secondly, locks which are not too malformed to be picked have lint which is in varying degrees stained and immature; in the tropical African cotton-growing territories this is bought separately at a much lower price. However, the total damage caused by internal boll disease remains undisclosed by market returns because so much of the affected cotton is not picked. Table 2 is therefore strictly a measure of the disorganization following infection at different ages, and underestimates the commercial loss sustained.

TABLE 2. *Effect of inoculation with* Nematospora *on final yield of seed cotton*

Mean air dry wt. in g. of seed cotton per lock in bolls inoculated at different ages.

	Age of boll in weeks at time of inoculation								
Treatment	2	3	4	5	6	7	8	9	Mean
N. gossypii	0·17	0·46	0·80	0·91	0·98	1·14	1·19	1·23	0·86
N. phaseoli	0·27	0·72	0·69	0·89	1·02	1·08	1·16	1·21	0·88
Mean	0·22	0·59	0·75	0·90	1·00	1·11	1·17	1·22	0·87
Control (sterile water)	0·50	1·10	1·15	1·07	1·09	1·10		1·25	

The analysis is based on fourteen bolls inoculated with each species of *Nematospora* on each occasion.

Results for week 1 are omitted, as it was impossible to separate seed cotton from carpels in this material.

Mean lock weight of forty-two uninoculated bolls drawn from the same material was 1·13 g.

Difference required between means of occasions at $P=0{\cdot}05$ is 0·08 g. and between means of species × occasions is 0·12 g. Means within each treatment which do not differ significantly are shown within brackets.

The effect of time of inoculation, and of the interaction of species × time of inoculation, are both highly significant, but there is no overall significant difference between the effects of the two species of *Nematospora*. In general *Nematospora* causes progressively less reduction in weight of seed cotton when inoculated into bolls of increasing age.

Unfortunately few bolls were inoculated with sterile water owing to a shortage of material, and they could not, therefore, be included in the analysis of variance. A separate analysis showed, however, that a significant difference existed between the mean boll weights of the treated and the control bolls at each of weeks

1–4. Furthermore, whereas the weights of bolls inoculated with sterile water at weeks 1 and 2 differed significantly from each other, and also from the weights on all other occasions, from week 3 inclusive there was no significant effect due to water inoculations, such boll weights differing neither amongst themselves nor from the mean of the forty-two uninoculated bolls.

Thus, although the effects upon weight of seed cotton of inoculations with sterile water are considerable in bolls up to 3 weeks of age, the effects of spore suspensions at those ages are by no means entirely due to the water contained in them. Furthermore, water inoculations have no effect on the weight of seed cotton in bolls of 3 weeks of age and older.

The weight of seed cotton ultimately produced by bolls inoculated at various ages as compared with the weight of seed cotton already laid down in those bolls at the time of inoculation is illustrated in Text-fig. 2. The two sets of data are not quite comparable, since the former material was air-dried, while the latter was oven-dried. After considering the humidities likely to have been encountered and the relationship between these and the moisture content of cotton seeds (Phillis & Mason, 1945), it seems unlikely that the error in the air-dry measurements exceeds 5%. The mean of the two *Nematospora* treatments has been used each week.

If a representative figure of 5 g. is taken as full size, this being the mean of the mature weights of groups VIII, IX and X plus all control bolls in groups III–VII, it will be seen that the increases in weight required to reach full size at weeks 2–8 inclusive are as follows:

4·5 3·5 2·9 2·1 1·2 0·8 0·2 g.

The increases in weight actually achieved following inoculation with *Nematospora* at those ages were:

0·6 1·0 0·9 1·0 0·5 0·6 0·2 g.

The increases consequently represent *percentage* achievements which improve as follows with increasing age at inoculation:

13 29 31 48 42 75 100%.

It follows, then, that the earlier the boll is inoculated, and hence the greater the amount of growth still to be made, the smaller is the proportion of this actually achieved.

(*d*) *Effect of inoculation upon seed weights and lint indices.* Ginning outturn (lint as percentage of seed cotton) was measured on bulked material from each treatment and date of inoculation, using a hand-roller gin, and seed weight (g. per 100 ginned seeds) and lint index (g. lint per 100 seeds) computed from these figures. These are three measurements commonly used in characterizing cottons. The proportion of 'full' seed was measured by treating seed for 2 min. with concentrated H_2SO_4, to remove fuzz, and separating 'full' from damaged seeds by floating off the latter in water. This is a common empirical test for germinating capacity. Bolls inoculated at 1 and 2 weeks of age were so badly damaged that 'lint' in the accepted sense did

not exist; in material from subsequent inoculations there was little difference in ginning outturns from the two *Nematospora* treatments and they have therefore been bulked in Table 3.

Table 3. *Effect of inoculation with* Nematospora *on ginning percentage, seed weight, lint index and percentage full seeds*

Age of boll in weeks at inoculation	Seed weight	Lint index	Ginning (%)	Full seeds (%)	Results as % of Age Group 9	
					Seed weight	Lint index
3	6·6	1·5	18·6	14·3	67·8	28·9
4	8·3	2·5	23·5	11·4	79·0	48·1
5	8·9	3·5	28·4	26·8	84·7	67·3
6	9·3	4·4	32·2	31·9	88·5	84·6
7	9·6	4·8	33·1	64·9	91·4	92·3
8	10·3	5·3	34·2	94·3	98·0	101·8
9	10·5	5·2	33·3	95·9	100·0	100·0
Uninoculated bolls	Not determined		33·0	97·5		

The table shows that the effect of inoculation upon all three measurements becomes progressively less serious as the age of boll at the time of inoculation increases, and that lint index is affected relatively more than seed weight or ginning percentage during the first 5 weeks of boll life. While seed weight is not very greatly affected in bolls inoculated after 5 weeks of age, the germinating capacity of such seed (as measured by percentage water sinking seeds) does not approach normal until week 8.

(*e*) *Effect of inoculation upon grade of staining.* The material from the experiment was graded, lock by lock, for intensity of staining, against a set of arbitrary standards used in routine assessment of stainer damage (see Pl. 15, fig. 2). The grades used were as follows: grade O, nil or only slight point of discoloration; grade I, localized staining, in patch, or spreading up suture line, or thinly over outer surface; grade II, heavy stain spreading over half lock, or lighter stain suffusing greater part of lock; grade III, whole lock, or greater part of it, heavily stained. In establishing these standards attention was directed to actual discoloration of the lint, and other characters such as lock size or lint maturity were as far as possible discounted. Table 4 shows the percentage of locks of each grade in each group of inoculations.

The number of control bolls was very small and they suffered some bacterial contamination. Their locks, moreover, were extremely difficult to grade, as the damage consisted more of a reduction in size and a breakdown of lint than of sheer discoloration of the material. The results for control bolls are thus irregular, but it will be seen that severe staining only occurred to any extent in those treated at 1–2 weeks of age.

In bolls inoculated with *Nematospora* a well-marked shift in the degree of staining was observed according to age of boll when inoculated, the results for the two

TABLE 4. *Staining of bolls inoculated with* Nematospora *at different ages*

Age in weeks at time of inoculation	Percentage of locks in different grades of staining											
	N. gossypii				*N. phaseoli*				Control (sterile water)			
	O	I	II	III	O	I	II	III	O	I	II	III
1	—	—	3	97	—	—	2	98	—	31	38	31
2	—	—	—	100	—	—	—	100	7	14	50	29
3	1	—	—	99	—	2	4	94	77	17	—	6
4	—	—	27	73	—	—	41	59	61	17	22	—
5	—	2	73	25	—	9	64	27	95	—	—	5
6	1	18	60	21	—	26	68	6	65	23	6	6
7	19	43	36	2	14	50	31	5	78	22	—	—
8	63	35	2	—	20	65	15	—	100	—	—	—
9	82	18	—	—	58	29	10	3	100	—	—	—
Not inoculated									92	7	1	—

species closely resembling each other. Generally speaking, the results indicate that under field conditions any boll severely stained will probably have been infected in the first 3–4 weeks of its life, any boll showing moderate staining will have been infected in weeks 5–7, and any bolls showing only light staining will have been infected in weeks 8–9. By allocating representative mean values of 10, 50 and 85% loss through staining to grades I, II and III respectively, and by combining data from both species of *Nematospora*, a single figure for staining loss can be estimated for each age group of bolls. This is shown in Text-fig. 1.

III. THE MECHANISM OF THE LETHAL EFFECT OF STERILE WATER UPON THE YOUNG BOLL

A feature of the 1934 experiment described above was the pronounced effect upon very young bolls of the control inoculations of sterile water. In 1933, using older bolls, such effects were not observed. When these lethal effects were first observed it seemed probable that they were due to plasmolytic action, and that the smaller the quantity of water introduced the less severe would be the damage resulting. Tests were made in 1938 to confirm this and exclude traumatic shock, or chance introduction of the methylated spirits used for sterilization, as possible causes.

Methods

Bolls of variety U4/096, protected from insect injury from the time of flowering, were treated as follows on 19 March 1938 when 15 days old:

(i) Inoculated with 0·2 c.c. sterile distilled water per lock, using the improved needle described in § II above. The boll surface was sterilized with absolute alcohol and allowed to dry before inoculation, and afterwards wiped clean with a sterilized swab before sealing with 'Durofix'.

(ii) Inoculated with 0·06 c.c. sterile distilled water per lock, using the same technique as above.

(iii) Dummy inoculation, the needle being inserted without introducing any fluid, but otherwise using the same technique as above.

(iv) Dummy inoculation, the boll being swabbed before and after with an excess of methylated spirits and the needle introduced with the boll surface still wet.

Five bolls were used in each treatment, one lock of each being left untreated as a control. All bolls were examined 2 weeks later.

Results

The results were extremely clear-cut. Treatments (iii) and (iv) had no effect at all, the embryos being normally formed and half to three-quarters grown and there being no secondary infection. In treatments (i) and (ii) the control locks were normal, as above, but in the treated locks the majority of the seeds were killed by treatment (i) and less than half killed by treatment (ii), the dead seeds having the outer epidermis stained brown and the lint matted on the surface, the inner seed coat proliferated and the embryo dead. There was a tendency for the badly affected locks to be invaded by secondary bacterial infections. The appearance of treated and normal locks is shown in longitudinal section in Pl. 15, figs. 6*a* and 6*b*.

In all, 79% of the seeds in locks inoculated with 0·2 c.c. sterile water were killed, and in a further 8% the embryos were poorly developed. In locks inoculated with 0·06 c.c. sterile water, only 33% of the seeds were killed and 7% were poorly developed. In all the control locks in these bolls, and in all the locks of the other treatments, seed development was normal.

The lethal effect is thus directly due to the water and, for any given age of boll, depends on the quantity introduced. In 1934, the inoculation of 0·2 c.c. per lock at 1 week, which had a very pronounced effect (mean lock weight at maturity 0·46 g.) represented the addition of about 170% of the water content of the developing seed cotton itself, while the same inoculation at 3 weeks, which had a negligible effect (mean lock weight at maturity 1·12 g., the same as uninoculated bolls) corresponded to only 9% of the water content of the seed cotton at the time.* The effect on 1-week-old bolls is thus explainable as a sheer swamping of the developing ovules; on the other hand, the effect of the same inoculation on 2-week-old bolls was almost as great (mean lock weight at maturity 0·54 g.), although here the inoculation represented only 20% of the water content. Moreover, in the 2-week-old 1938 material the introduction of 0·06 c.c. per lock, representing only 6% of the water content of the seed cotton, killed 33% of the seeds. It seems probable, therefore, that some change in the permeability of the seed-coat tissues is concerned in the sharp drop in susceptibility to water which occurs between 2 and 3 weeks of age. Much of the effect could be avoided by using sufficiently small dosages, and a simple apparatus was devised, in collaboration with Mr F. R. Parnell, whereby doses of approximately 0·006 c.c. could be fairly accurately delivered by using a micrometer screw bearing on the plunger of an ordinary 2 c.c. syringe. This method has recently been used by Wickens (1947) with good results.

* I am indebted to Dr R. C. Rainey for this point.

IV. The comparative development of the effects of inoculations of living spore suspensions and toxic extracts of *Nematospora gossypii*

The first suggestion that the staining characteristic of *Nematospora* is due to a modification of the protoplasm of the central canal of the lint hair was made by Marsh (1925). This was confirmed by the 1933 experiments at Barberton, which suggested that staining is due to the coagulation and discoloration of the protoplasmic contents of the developing lint hair following death due to a toxin liberated from *Nematospora* (Pearson, 1934). Further observations in 1934 and 1935 on the mode of action of the fungus are described below.

1934 *Experiment*

Methods. Much lower concentrations of inocula were employed than in 1933, the treatments being (i) a spore suspension prepared by shaking up the whole surface growth of an 11-day-old, potato-dextrose-agar culture of *N. gossypii*, in 20 c.c. sterile water and diluting 2 c.c. of this in 30 c.c. sterile water; (ii) a toxic extract obtained by autoclaving half this spore suspension; (iii) sterile distilled water. 0·1 c.c. of each was inoculated into separate locks of the same boll, the remaining locks being left as controls. Bolls were inoculated on 12 April 1934 when 5 weeks old, and duplicate lots of ten examined after 6 and 14 days.

Results. When examined, the bolls showed seeds and embryos fully formed, lint partly thickened and no free moisture. Locks inoculated with living *N. gossypii* showed lint staining on the majority of seeds after 6 days, increasing in intensity after 14 days, more pronounced at the base of hairs, but also occurring discontinuously, especially along the suture line. The protoplasm showed through the lint wall as a yellow-brown coagulated granular mass broken into short cylinders. The seeds were unaffected except for brown spotting of the epidermis. Copious hyphae and immature sporangia occurred throughout the lock after 6 days, but free spores were not liberated until after 14 days.

Sterile-water inoculations resulted only in occasional specks of discoloration immediately below puncture.

Toxic extract produced a distinct reaction, lint being stained on the suture line, and also at the hair bases, on up to half the seeds of the lock, but the effect was less marked after 14 days than initially. No trace of any organism appeared.

The reaction to the toxic extract in this experiment was thus very much less marked than in the previous year.

1935 *Experiment*

Methods. To follow the progressive effects of different inocula in bolls of different ages, samples of bagged bolls from flowers of 20–22 February 1935 were separately inoculated when 2, 4 and 6 weeks old, subsamples being examined at intervals after inoculation and oven-dry weights of seed cotton determined. The inocula used were: (i) a spore suspension made by shaking up a 10-day-old, potato-dextrose-agar culture of *N. gossypii* in 150 c.c. sterile water; (ii) a toxic extract prepared by macerating a parallel culture in 150 c.c. sterile water, centrifuging, filtering and sterilizing at 35 lb. pressure; (iii) sterile distilled water. Each lock was inoculated with 0·2 c.c.

TABLE 5. *Development of reaction to sterile water and spore suspensions or toxic extracts of* Nematospora gossypii

Age of boll in days at inoculation	Inoculum	General reaction	Incubation period in days before examination			Recovery of fungus
			8	21	35	
14	Spore suspension	Immediate, severe, some abortion	Lint often reduced to papery membrane closely enfolding collapsed seeds	Some aborted, 1 to 1·5 cm. diam., same condition as at 8 days; others superficially healthy, 2 to 3 cm. diam., lint broken down, seeds brown, boll wall and inner seed coat proliferated, embryo dead	Aborted bolls dry, brittle; others with locks blotchily stained and kidney-like, boll wall heavily stained and proliferated, seeds internally proliferated and dead	Difficult to find except as traces
	Toxic extract	Intermediate between spore suspension and sterile water	Seed coat severely discoloured, lint locally reduced to papery membrane, or sodden, yellowed; but unaffected at extremities of locks	Practically all seeds brown, dead, internally proliferated, closely enfolded by dirty grey or brown, sodden, matted lint	All seeds dead, appearance as after 21 days	Nil
	Sterile water	Immediate, severe, but no abortion	Light to intense brown staining of seed, latter state accompanied by sodden appearance of lint	Embryos aborted in several seeds, on which lint was unthickened, sodden and matted	Great irregularity in size; often all seeds dead, internally proliferated, brown; lint matted but not deeply stained	Nil
29	Spore suspension	Gradual, uniform	Some proliferation and staining inside boll wall; seeds apparently unaffected; lint with matted, darkly stained immature neps or straw yellow to light brown stain of fibres	Massive proliferation developed inside boll wall, especially up suture lines, to which stained lint firmly adhering—origin of 'webbing'	—	Hyphae and spores in large quantities after 3 weeks
	Toxic extract	Slight	Small streaks of stained lint	Streaks of stained lint	—	Nil
	Sterile water	Nil	No effect	No effect	—	Nil
42	Spore suspension	Slight	Light yellowish stain of lint hairs, severer proximally, between central seeds of lock, on suture lines and inner lock faces	—	—	Traces
	Toxic extract	Very slight	Small specks of stained lint	—	—	Nil
	Sterile water	Nil	No effect	—	—	Nil

Results. The development of the reaction to the three inocula is shown in Table 5, and the effects as measured by oven-dry weights of seed cotton are shown in Table 6.

TABLE 6. *Development of seed cotton in bolls inoculated at different ages with sterile water and spore suspensions and toxins from* Nematospora gossypii

Mean weight of seed cotton in g. per lock at intervals after inoculation.

Age in days when inoculated	Inoculum	Incubation period (days) 8	21	35	Diff. required for significance at $P=0{\cdot}05$
14	Spore suspension	0·10	0·09	0·07	0·01
	Toxic extract	0·13	0·13	0·25	
	Sterile water	0·20	0·29	0·40	
29	Spore suspension	0·48*	0·57	—	0·23
	Toxic extract	0·55*	0·85	—	
	Sterile water	0·59*	0·93	—	
42	Spore suspension	0·83	—	—	
	Toxic extract	0·98	—	—	
	Sterile water	0·97	—	—	

* Incubation period only 7 days.

On account of the different levels of variance, the analysis was carried out separately for the material inoculated when 2 weeks old, and for the remainder. The effects on 2-week-old bolls were highly significant, the only results not differing at the 5% point being those from material examined 8 and 21 days after inoculation with *N. gossypii* spores and with toxic extract. All other results differed significantly from each other. They showed the profound effects of all the inocula on young bolls, those effects being greater, with growth more abruptly suspended, in the case of the living fungus than in that of sterile water, with the toxic extract occupying an intermediate position. In the rest, i.e. bolls inoculated at 4 and 6 weeks, the immediate effects were much less severe, there being no significant difference between different treatments after 1 week. But while in the case of living spores the further growth of 4-week-old bolls was arrested, there being no significant increase in lock weight between 7 and 21 days after inoculation, the sterile water and toxic extract had little or no effect, the locks continuing to make significant increases in weight.

V. The effect of punctures by uninfected *Dysdercus*

The condition which follows the inoculation of young bolls with sterile water is strikingly similar to that developed as a result of feeding by stainers (*Dysdercus* spp.) which are not infected by pathogenic fungi or bacteria. This condition was first described by Morrill (1910) and subsequently by Nowell (1917) and Laycock (1925). Attention was particularly drawn to it in 1934–5, when routine tests were being made of the extent to which *Dysdercus* spp. collected on wild hosts were infected by *Nematospora* spp. and bacteria. These tests were largely carried out

using young bolls (less than 4 weeks old, but exact details of their age not recorded), and the notes which follow refer to those in which no trace of *Nematospora* or of pathogenic bacteria could be found.

Only seeds directly punctured by the stylets showed any abnormality. In these, the embryo was shrivelled and browned and the inner seed coat much proliferated, forming in extreme cases a floury mass of semi-vacuolate, roughly spherical cells, loosely bound together and containing numerous granules. Staining in this tissue was confined to stylet tracks, along which the protoplasm lining the vacuolated parenchyma cells was stained yellow and tended to coagulate.

Lint hairs, especially for some distance round the point of puncture, were unthickened, completely collapsed and difficult to tease out as individual fibres, becoming matted together into a sodden, rather slimy, membranous mass, which later sometimes became pale brown, though frequently remaining semi-transparent. Eventually this membrane could be peeled off the seed. The inside of the young boll wall frequently showed raised, opaque, spongy, white or brownish proliferating masses surrounding the puncture; older bolls showed only the usual circular, slightly raised 'glassy' areas.

The bolls displaying these effects were all less than a month old. Older ones, with fully formed embryos, seem able to withstand non-infected punctures without ill effects on the lint and with effects on the seeds which depend on the amount of feeding which has occurred. Nowell (1917) makes the same observation, and the whole matter has recently been carefully investigated by Wickens (1947), who notes, in an interim report, that the effect on the lint is barely perceptible in bolls exposed, when more than 4–5 weeks old, to uninfected stainers.

The condition in young bolls following attack by uninfected stainers thus differs from that accompanying infection by *Nematospora* chiefly in the much more localized effects, which are confined to seeds directly punctured, and in the limited staining of the lint, the dark yellowish green or brown colour typical of *Nematospora* in young bolls not developing. This distinction has been most clearly recognized, as it appears in bolls which reach maturity, by Laycock (1925), who describes direct injury due to the feeding of sterile *Dysdercus* as well-defined, localized, brown patches of lint, in bad cases the whole lock being shrunken, corrugated, brown and matted. In contrast, he states, locks punctured by field-collected infected stainers show similar effects at the point of puncture, but starting from this point the greater part of the lint is often stained a light yellow, this staining being diffused, not localized. Wickens (1940, 1942) similarly regards diffuse yellow staining as a criterion of the presence of *Nematospora*.

If, however, bolls are severely attacked at an early age by either infected or non-infected *Dysdercus*, the boll contents in either case can be so completely disorganized that it is difficult to distinguish diffuse yellow staining of the lint. A further difficulty in recognizing the cause of internal boll disorders is that the staining and disorganization caused by *Nematospora* in young bolls often

extends much beyond the region where the fungus can be found. It seems as if the extensive tissue destruction which it causes eventually itself inhibits the further growth of the organism. Failure to find *Nematospora* in severely affected bolls is therefore not necessarily an indication that the damage has been directly caused by bug feeding.

VI. Discussion

The effects of *Nematospora* upon the developing boll closely depend on the age at which the boll becomes infected, and are largely explicable in terms of the stage of development then reached by the various tissues. They are twofold: first, an interference with the growth of the whole boll, and secondly, a localized effect upon the lint.

In the first 2–3 weeks* of life there is rapid growth of the carpel walls, ovules and endosperm and in the length (but not thickness) of lint. The tissues of the seeds, in particular, are delicate and the embryo is still extremely minute (Balls, 1915). It is in this stage that the seed is so susceptible to inoculations of sterile water, probably because the tissues are then more readily permeable, thus accounting for the death of the embryo by plasmolysis. The death of the other tissues in the seed, including the lint cells, follows eventually, although not immediately, for the inner seed coat continues proliferation after the embryo has died.

The effects of sterile water are confined to those seeds coming into direct contact with relatively large volumes of water; where toxic extracts or living spore suspensions are conveyed in the same quantity of fluid the results upon young bolls are much more extensive and severe. Some kind of toxic action is clearly operative here, for embryos are killed without any penetration of the seed by the needle used or by the fungus mycelium.

Once the ovules have been killed, supplies to the boll are either completely cut off, so that it sheds, or reduced, so that it aborts or is malformed and undersized.

By 4 weeks of age, however, when the seed is full-sized and the rapidly growing embryo is protected by the thickening and hardening of the inner seed coat, the viability of the embryo is no longer affected by the fungus, or by plasmolysis.

Meristematic activity is confined to the first 4 weeks of boll development, and it is only during this period, before the tissues are fully differentiated, that the mechanical effect of needle or insect puncturing, or the irritant stimulus of fungi or toxins, produces proliferation.

The influence of the fungus upon the lint hairs is evidently connected with their mode of development; they arise as thin-walled cells in the epidermis of the seed and reach their full length in the first 4 weeks of boll development; only then does secondary thickening of the cell wall start, and as this continues the protoplasm which initially fills the lumen becomes vacuolated until, about the sixth or seventh week, it is reduced to a thin lining. When bolls are infected during the first 3 weeks of their

* In the remarks which follow the time scale relates to bolls which begin to split at between 9 and 10 weeks.

life, the lint is killed, either directly or following the death of the embryo, and it is presumably because at this stage the lint is still very thin walled that it partly disintegrates and becomes matted together to form the papery membrane so characteristic of this stage of infection. At the critical point when the boll is large enough and the seed sufficiently well developed for infection not to produce abortion, but when the carpel walls are still young enough to be stimulated to meristematic activity and the lint has not become secondarily thickened, the phenomenon of 'webbing' is induced, due to the unthickened lint sticking to the proliferations arising from the carpels, and thus being stretched across the open boll after these have reflexed.

The marked change in the character of the damage done to lint in bolls more than 4 weeks old is due partly to the secondary thickening of the cell wall and partly to the vacuolation of the protoplasmic contents. Even if the lint cell is then killed, the thickening cellulose walls do not break down and thus the hairs do not mat together but remain discrete, though only partially mature. The protoplasm acquires a coagulated, granular texture and a characteristic yellowish or greenish brown colour; the older the boll, and hence the more vacuolated the hair cell, the thinner is the protoplasmic lining that can be so affected, and consequently the lighter is the degree of staining induced. It seems possible also that as the cell walls thicken, they give additional protection to the protoplasm within.

The discoloration of the protoplasm, however, is not a mere post-mortem effect, for in young bolls where the embryos have been killed by water inoculations or by direct insect feeding, the lint is not densely stained—although it obviously must be killed—but becomes sodden and parchment-like. The characteristic discoloration is evidently closely connected with the fungus, for it is produced by inoculation of either the living organism or the sterile extract.

All the changes which take place after the first week in the developing cotton seed—the growth of the embryo and its replacement of the endosperm, the sclerotization of the seed coats, the secondary thickening of the lint hair—are accompanied by a reduction in water content and, consequently, there is a close correlation between the moisture content of the developing seed cotton and the degree of staining following infection (see Text-fig. 1).

There is, moreover, a certain amount of 'free' fluid in the developing boll, particularly between 1 and 6 weeks of age, forming a film over the lint hairs. This acts as a medium for the development of *Nematospora*, which grows amongst the lint hairs, without penetrating them to any great extent. It is noticeable that inoculated locks are most severely stained along the suture line and over the outer faces, perhaps because these are the natural channels along which fluid injected under slight pressure would travel. But the same distribution of staining is found in naturally infected bolls, and, while the stainer's salivary fluid might also, conceivably, be injected under sufficient pressure, there may be some movement of fluids within the boll cavity carrying the fungus or its products. This would further explain

the limitation and localization of staining in older bolls where there is less free fluid.

Rainey (1940), reporting on the changes in the chemical constitution of the developing boll, remarks that reducing sugars fall from nearly 50% of total dry matter at 1 week old to less than 1% in the mature boll, providing a possibly suggestive parallel with the changes in reaction to *Nematospora* reported by the writer. The theory is attractive, but the degree of damage caused by *Nematospora* is not necessarily a measure of the vigour of its growth—indeed, the fungus is rather difficult to find in the youngest, most severely damaged bolls and spreads most vigorously in bolls of medium age. The explanation of the connexion between age of boll and reaction to *Nematospora* which is given here in terms of tissue development seems simpler and therefore preferable, but clearly investigations of the growth and behaviour of *Nematospora* in relation to its nutrition in the developing boll are of first-rate importance.

The evidence regarding the mechanism of the lethal action of *Nematospora* is rather conflicting. The fungus being weakly parasitic and incapable of attacking cellulose, its widespread effects were originally attributed to toxins. Preliminary experiments supported this hypothesis, sterile extracts of *N. gossypii* having produced toxic effects on 6-week-old bolls in 1933, but only slight effects were observed in 1934 on 5-week-old bolls, and negligible effects on 4- and 6-week-old bolls in 1935, although there was a well-marked reaction in 2-week-old bolls that year.

Some explanation may be given by the physiological age of the bolls used, and by the dosage. The 1933 bolls were from flowers of 24 April and would have been developing very slowly in the low temperatures then ruling. That they were, in fact, physiologically younger than normal 6-week-old bolls is shown by their strong reaction to living *Nematospora*. The toxic extract was prepared from a 22-day-old culture, whereas those used in 1934 and 1935 were from cultures 11 and 10 days old respectively. Cultures 22 days old are ceasing active growth (Frazer, 1944), due presumably to 'staling', and if the toxic products involved in staling are responsible for the death of the lint hair, the ages of the cultures used may partly account for the different degrees of reaction observed.

These results are highly relevant to the incidence of damage due to internal boll disease. Clearly, the older the bolls at the time they are attacked by the insect vectors of *Nematospora*, the less intense will be the damage resulting. In the majority of the cotton-growing countries in Africa the first immigrant *Dysdercus* do not enter the fields until shortly before the bolls begin to open. Frequently the initial immigration is on a small scale, and large numbers of vectors are not available until 3–4 weeks later when breeding has taken place, and the later nymphal stages of the new generation appear. If the variety grown has a high initial rate of flowering, so that the whole of its effective crop reaches maturity over a relatively short period, and if sowing dates, conditions of cultivation and freedom from other pests or diseases are favourable, then the great majority of the bolls pass through their

susceptible phase before there are sufficient vectors bred in the crop to give a high rate of punctures per boll.

These conditions apply to much of the rain-grown cotton of the southern African savannah areas, where there is a single rainy season and where early maturing, strongly sympodial, vigorously fruiting types of cotton descended from the Barberton U 4 strain are grown. These habitually produce the greater part of their effective crop from the earliest part of the flowering curve, and although the infestations of *Dysdercus* may eventually become very large due to breeding in open bolls, the bulk of the crop escapes serious damage, resulting in the apparent anomaly at the end of the season of a large stainer population co-existing with a clean, well-opened crop.

The reverse occurs either when stainer immigration is early, or boll production is retarded. The former occurs in Northern Rhodesia, where cotton is invaded by *Dysdercus superstitiosus* F. whilst still in its main flowering phase (Bebbington & Allan, 1931 et seq.), and to some extent in the Eastern Province of Tanganyika where *D. intermedius* Dist. has similar habits (Bebbington & McKinstry, 1943 et seq.). Bolls are attacked whilst still very young and highly susceptible, not only to the fungi but also to the mechanical effects of feeding, and extremely severe damage results. Much the same effect may be noted where cotton crops in very different stages of development are grown in close proximity, giving rise to chance infiltration into young crops by vector populations attracted to or originating in older ones. The second condition, the retardation of boll production, is found where the incidence of another pest, such as American bollworm, *Heliothis armigera* Hübn., or red bollworm, *Diparopsis castanea* Hmps., causes heavy losses of flower buds and young bolls, leading to a prolonged flowering period. This condition arises particularly in Nyasaland (Pearson & Mitchell, 1945). Bolls formed from the later flowers pass through their susceptible phase exposed to the attacks of vectors attracted to and bred on the open bolls surviving from the early part of the flowering, and consequently sustain serious damage.

The writer's thanks are due to two former colleagues at the Cotton Experiment Station, Barberton; to Mr W. L. Fielding for the photography and to Dr R. C. Rainey for criticizing the first draft.

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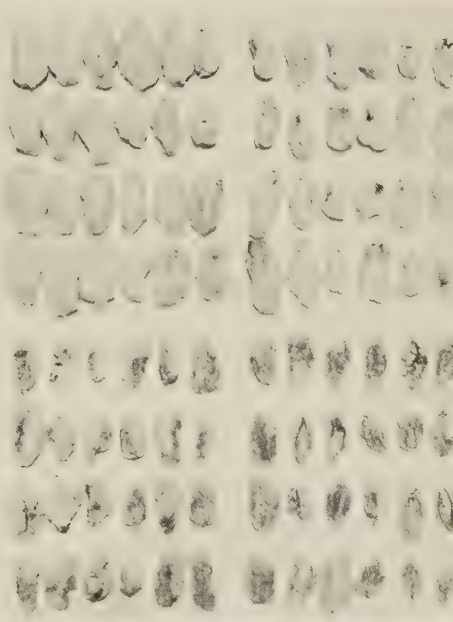

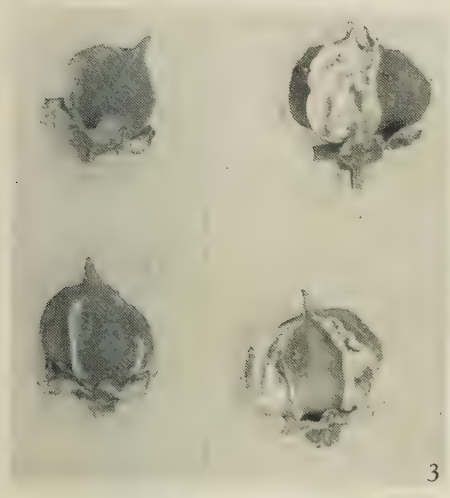

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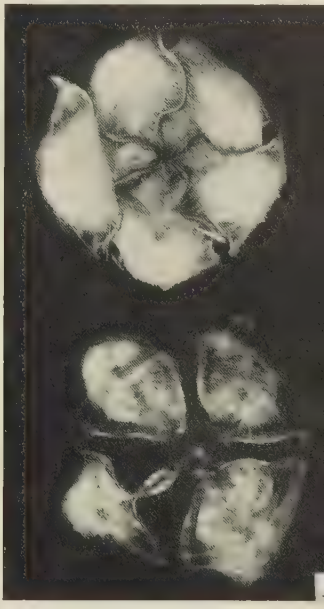

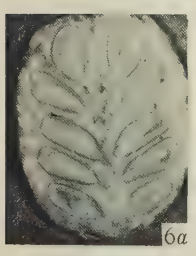

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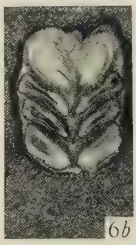

6*b*

PEARSON—*The development of internal boll disease of cotton*

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EXPLANATION OF PLATE 15

Fig. 1. Bolls inoculated at different ages with *Nematospora*. Age in weeks at time of inoculation shown by figures; 'nil' group, uninoculated bolls. In each age group: top, bolls inoculated with *N. phaseoli* Wingard; middle, bolls inoculated with *N. gossypii* Ashby & Nowell; bottom, bolls inoculated with sterile water.

Fig. 2. Arbitrary grades of staining. Top left, grade O; top right, grade I; bottom left, grade II; bottom right, grade III.

Fig. 3. Bolls inoculated when 1 week old. Left: *Nematospora gossypii*. Right: *N. phaseoli*.

Fig. 4. Bolls inoculated when 2 weeks old. Top: *Nematospora gossypii*. Bottom: *N. phaseoli*.

Fig. 5. Bolls inoculated when 4 weeks old. Top: *Nematospora gossypii*. Bottom: *N. phaseoli*.

Fig. 6*a*. Longitudinal section of lock from normal 31-day-old boll, showing seeds embedded in lint, dark outer seed coat and thicker lighter inner seed, and embryo developed to fill most of embryo sac.

Fig. 6*b*. Longitudinal section of lock from 31-day-old boll, inoculated 16 days previously with 0·2 c.c. sterile distilled water per lock, showing top pair of seeds unaffected, central and lower seeds collapsed, with lint disintegrating, inner seed coat proliferating to fill embryo sac, and embryo destroyed.

(Figures 1 and 2 much reduced, 3, 4 and 5 slightly reduced, and 6*a* and 6*b* approximately natural size.)

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OBSERVATIONS ON TAKE-ALL AND EYESPOT DISEASES OF WHEAT IN YORKSHIRE

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(With 1 Text-figure)

Wheat crops were surveyed in Yorkshire from 1944 to 1946 on farms where crops were reported to be unsatisfactory. Take-all (*Ophiobolus graminis* (Sacc.) Sacc.) and eyespot (*Cercosporella herpotrichoides* Fron.) were both found to be present; the latter is the most serious trouble on the better wheat lands.

The variation in the incidence of these diseases on selected farms during the three seasons has been compared.

The effect of rotation has been examined; both diseases were found to be encouraged by too frequent cropping with wheat or barley. The incidence of disease in wheat crops following a 1-year seeds ley was found to be influenced by the nurse crop used to undersow the seeds, and also by the time at which the ley was ploughed up. Oats were found to be preferable to either wheat or barley as a nurse crop, and in 1946 late ploughing considerably reduced the amount of eyespot disease present in the following wheat crop.

A 1-year ley is considered to be of too short a duration to ensure the disappearance from the soil of *Ophiobolus graminis* and *Cercosporella herpotrichoides* surviving from the previous crop, and a period of 2–3 years is suggested as being desirable.

Introduction

Wheat crops principally in the East Riding of Yorkshire were surveyed in the month prior to harvest from 1944 to 1946 on farms where crops were reported to be unsatisfactory. The amount of take-all (*Ophiobolus graminis* (Sacc.) Sacc.) and eyespot (*Cercosporella herpotrichoides* Fron.) was determined in samples taken at random. The distribution of eyespot in certain English and Welsh counties and in Scotland has been investigated by Glynne (1942, 1946), but the prevalence of this disease in Yorkshire has been little studied.

Methods

The method used in surveying the crops was based on that described by Buddin & Garrett (1944). Ten samples, each consisting of a double handful of straws, were taken at random in a diagonal traverse of the field. The Whitehead tillers caused by the take-all fungus were counted and removed from the sample, and the number of tillers in the remainder with severe eyespot lesions was determined. In the following tables these figures are given as the percentage take-all and percentage eyespot respectively.

Results

(*a*) *Distribution of eyespot*

Fig. 1 shows the location of fields having more than 20% of the tillers attacked by eyespot. These were mainly in the Holderness area, at scattered places on the plain

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of York, and on the warp land adjoining the Humber. In the heavy land of the Holderness area, where wheat is one of the main crops, it is a common practice to take a wheat crop three times in 5 years. A typical rotation on farms where eyespot is severe is as follows: fallow—wheat—seeds—wheat—peas—wheat.

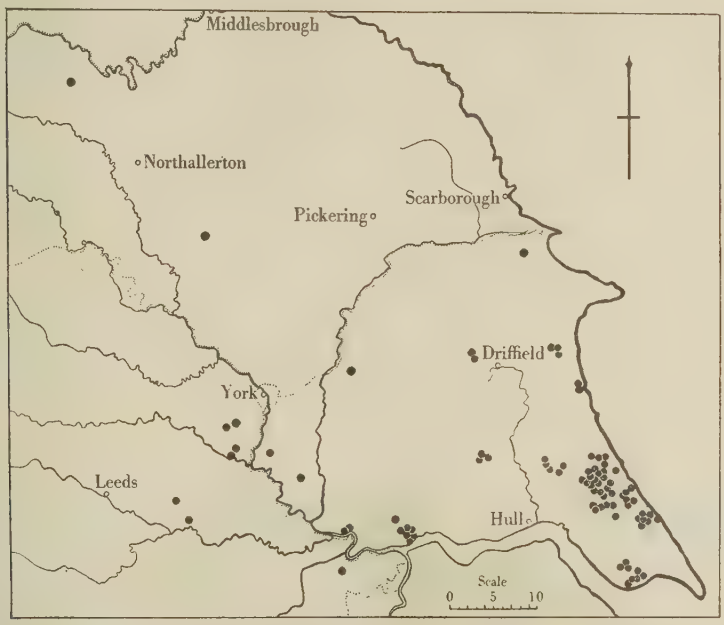

Fig. 1. Distribution of fields in Yorkshire with more than 20% of the tillers attacked by eyespot. • Infected field.

(*b*) *Seasonal variation*

The variation in the amount of take-all and eyespot from year to year on six farms in the East Riding is given in Table 1. Only those crops which were growing in the following sequence have been included: wheat or barley—non-cereal crop—wheat.

TABLE 1. *Incidence of take-all and eyespot from* 1944 *to* 1946 *on six farms in the East Riding of Yorkshire*

		Take-all		Eyespot	
Year	No. of fields	% fields infected	% take-all	% fields infected	% eyespot
1944	13	30	3	100	55
1945	19	84	6	100	27
1946	19	26	3	100	50

In 1945 a higher proportion of the fields was found to contain Whiteheads, and

the degree of infection was also greater than in 1944 and 1946. Eyespot was present in all fields each year, but the degree of infection was much less in 1945.

(*c*) *Effect of rotation*

The effect of lengthening the interval between successive crops of wheat and barley is shown in Table 2.

TABLE 2. *Effect of the length of the interval between susceptible crops on the incidence of disease*

Previous cropping	No. of fields	% take-all	% eyespot
Susceptible crop in previous year	14	20	36
1 yr. interval between susceptible crops	101	4	43
2 yr. interval between susceptible crops	18	1	34
3 yr. interval between susceptible crops	17	0	6

Take-all is more severe in wheat following wheat or barley. A year under a non-susceptible crop considerably reduces the amount of take-all, and after 2 years the disease is almost absent. A similar period is not sufficient to reduce eyespot to the same level, and an interval of at least 3 years is required.

(*d*) *Effect of a seeds ley*

A considerable proportion of the wheat crops in Yorkshire follows a 1-year seeds ley, consisting of a mixture of clovers or of grasses and clovers. Some factors which influence the incidence of disease in the following wheat crop are considered below.

(1) *Influence of the nurse crop.* In Table 3, the effect of undersowing a 1-year seeds ley in oats, wheat or barley on the incidence of disease in the following wheat crop is compared.

TABLE 3. *Effect of the seeds nurse crop on the incidence of disease in the following wheat crop*

Crop sequence	No. of fields	% take-all	% eyespot
Oats—seeds—wheat	16	1	8
Barley—seeds—wheat	32	6	36
Wheat—seeds—wheat	20	5	44

There is considerably less disease present in the wheat after seeds undersown in oats than after seeds undersown in wheat or barley. The oat crop is known to be highly resistant to both take-all and eyespot. Wheat or barley as a nurse crop has very little effect on the amount of take-all, but eyespot is more severe in wheat following seeds undersown in wheat than when it follows seeds undersown in barley. It has long been recognized that a nurse crop can exert an effect on the following wheat crop. An observer (Anon. 1837) in Essex reports that a wheat crop following a 1-year seeds ley undersown in oats has been reputed to yield

several bushels more than one undersown in wheat or barley. Although disease is not mentioned, the presence of take-all and eyespot may well have been the explanation of such an effect.

(2) *Effect of time of ploughing up a ley.* The time of ploughing up a ley may influence the amount of disease present in the following wheat crops. In 1946 three fields all sown at the same time but part of which had received different treatment during the summer were surveyed. Half of each field had been bastard fallowed, whilst the other half had been ploughed immediately before sowing. The results are given in Table 4.

TABLE 4. *Effect of early and late ploughing a 1-year seed ley on the incidence of take-all and eyespot in the following wheat crop*

	Incidence of disease			
	Early ploughing		Late ploughing	
Field	% take-all	% eyespot	% take-all	% eyespot
A	0	60	0	12
B	0	52	13	4
C	0	74	0	37
Average	0	62	4	17

The amount of eyespot is reduced very considerably by the late ploughing, but this could not be recommended as a control measure owing to the poor weed control obtained. These fields were full of docks and thistles, whilst the bastard fallowed portions were almost free from weeds. The plant density was less in the late-ploughed halves, and this in itself would help to control the disease. A similar effect is described by Russell & Watson (1940) where summer fallowing after the first cut of ley increased the survival of wheat plants as compared with taking two cuts of ley.

(3) *Effect of the length of the ley.* The majority of crops surveyed have been following a clover or clover and grass ley of 1 year's duration. A few wheat crops growing after leys of 2 or 3 years' duration have been examined, and in no instance has any appreciable amount of take-all or eyespot been found. In fact, some of the crops examined have been the healthiest seen in Yorkshire.

The disease present after a 1-year ley would appear to be a legacy from the nurse crop except in those instances where weed grasses perpetuate the disease from year to year. The duration of a 1-year ley is insufficient for the dying out of the disease organisms, a period of at least 2–3 years being desirable to complete the process.

The writer wishes to express his thanks to Dr Mary Glynne for much help and advice; to Dr Millard; his former colleagues of the Department of Agriculture in the University of Leeds and members of the staff of the East Riding W.A.E.C. for much assistance during the progress of the work; and to Mr A. Beaumont for help in the preparation of the manuscript.

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INVESTIGATIONS ON THE GOUT FLY (*CHLOROPS PUMILIONIS* BJERK.) IN DEVON AND CORNWALL

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(With 5 Text-figures)

Field observations from 1943 to 1946 on attacks by gout fly on wheat in south-west England show that there are two generations of the fly during the year, the first emerging in May from October-laid eggs and the second emerging in late July and early August. The first generation severely damages spring wheat and may also attack very late sown and backward autumn wheat; wheat not above ground before 20 October escapes attack by the second generation. In south-west England, the optimum period for drilling wheat to escape gout-fly damage is mid-October. In this area, both generations of gout fly show a marked preference for wheat; attacks on barley are negligible.

Introduction

Since the paper by Frew (1924) on the gout fly of barley and a general account by Imms (1925) embodying the results of Frew's work, comparatively little attention has been paid to this pest. Although it is common on barley in many parts of Britain it occurs as a major pest of wheat only in the south-west. Frew records a slight attack on wheat, a few tillers only being affected. The first report of a severe attack was from Bodmin (Hodson, 1930), and this was followed subsequently by other records in Devon and Cornwall. During recent years many complaints have been received from farmers in this area of fields of wheat turning yellow with many plants dying; visits to such fields almost invariably revealed an attack by gout fly. In several fields the attack was so severe that the whole crop had to be ploughed under. In addition, many fields at harvest time showed large numbers of retarded tillers and poorly developed heads, and in some crops it was observed that a high proportion of small grain resulted. As little was known of the behaviour of gout fly as a pest of wheat the investigations described in the present paper were undertaken.

Effect of sowing date

Preliminary observations during 1943

A general survey during February and March, when damage could easily be observed, showed this pest to be widespread throughout the province, being more serious in Cornwall than in Devon. However, late-drilled fields of winter wheat which were backward showed no signs of attack. As it thus seemed likely that the date of drilling affected incidence of attack, particularly by the overwintering generation, farmers were asked for particulars of fields drilled to winter wheat.

Data were received on drilling dates ranging from 1 to 29 October, and twenty-one fields were selected for close examination.

Observations during 1944 *and* 1945

Winter wheats. During February 1944 the fields were visited, and in sixteen the percentage of plants attacked was ascertained. The symptoms were obvious at this time (Fig. 2) and were the same as those described by Frew (1924) for barley.

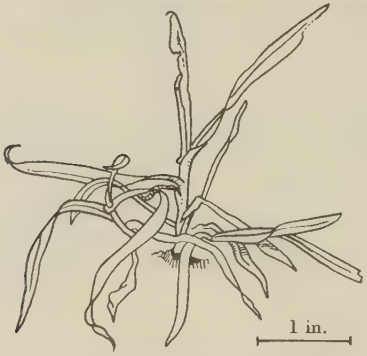

Fig. 1. Early stage of attack of wheat plant by gout fly. Drawn 8 January.

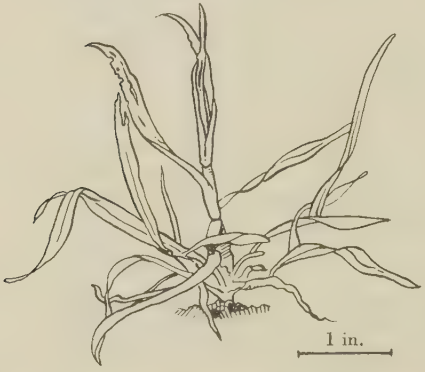

Fig. 2. The same plant as in Fig. 1, drawn 5 February on the same scale and from the same view point.

Fig. 3. A. The same plant as in Figs. 1 and 2 drawn on 9 March. Note the growth of healthy tillers. B. The same plant on 16 March. Before drawing all the healthy tillers were cut off, leaving only those attacked by gout fly.

Figs. 1–3 are drawings made by Staniland (1946) showing the development of an attack by gout fly on the same wheat plant from the first symptoms in early January to an advanced stage in March.

Counts were taken from ten sites divided into two sets of five. Each site consisted of three drills each 24 in. in length, and defined by a frame 24 × 12 in. laid lengthwise along the drills.

In October 1944 drilling dates were obtained in the same way as in the previous year; in February 1945 counts were again obtained. At this time twenty fields were selected, two sets of ten sites being counted instead of two sets of five as in 1944. The results are given in Tables 1 and 2. The dates of drilling, which are also given in these tables, do not represent a uniform series. Such a series would be very difficult to obtain, as climatic conditions are so variable; often periods of several days occur which are quite unsuitable for the field drilling of cereals.

TABLE 1. *Relation of date of drilling of winter wheat in Cornwall to intensity of attack by August generation gout fly, February* 1944

Field no.	Drilling date	Total plants	Mean % attack	S.E. as % of mean
1	1. x. 43	443	90·3	± 23·9
2	2. x. 43	457	82·3	± 12·0
3	4. x. 43	498	84·7	± 8·4
4	4. x. 43	349	65·3	± 14·2
5	5. x. 43	449	41·9	± 14·5
6	8. x. 43	398	6·0	± 5·0
7	8. x. 43	558	22·8	± 4·6
8	12. x. 43	571	5·0	± 3·1
9–16	16–29. x. 43	2869	0	0

TABLE 2. *Relation of date of drilling of winter wheat in Cornwall to intensity of attack by August generation gout fly, February* 1945

Field no.	Drilling date	Total plants	Mean % attack	S.E. as % of mean
1	28. ix. 44	1315	36·5	± 18·5
2	3. x. 44	1103	35·4	± 27·2
3	5. x. 44	1260	15·9	± 28·3
4	5. x. 44	1130	16·8	± 25·4
5	6. x. 44	1357	13·8	± 20·0
6	6. x. 44	1186	26·1	± 18·6
7	6. x. 44	956	17·9	± 20·5
8	6. x. 44	1252	10·5	± 26·5
9	7. x. 44	1357	2·8	± 37·9
10	7. x. 44	689	16·3	± 18·6
11	7. x. 44	1262	13·0	± 28·7
12	9. x. 44	825	4·4	± 55·2
13–20	9–27. x. 44	7400	0	0

In order to obtain a uniform series of sowing dates experimental plots were laid down. During the autumn of 1944 ten duplicated plots were sown at 4-day intervals, each approximately 7 yd. square; the sowing dates ranged from 25 September to 1 November inclusive. In February 1945 plant counts were taken using the same frame as for the field counts; five sites were taken at random from each plot. The results are given in Table 3.

In late July 1944, further counts were taken from the sixteen fields shown in Table 1, to determine the percentage attack by the May generation. Two sets of ten sites were taken, each site being 1 ft. length of a single drill. The tillers were severed

close to the ground, and the two sets bulked and brought back to the laboratory for counting (Table 4). In July 1945 similar counts were taken from the twenty fields indicated in Table 2; the results are tabulated in Table 5. Counts of the attack by the May generation were also recorded from the plots, two 1 yd. square sites per plot being taken (Table 6).

Spring wheat. Spring wheat is not widely grown in the south-west. The results obtained from six fields during July 1944, and from three fields during July 1945, are given in Tables 4 and 5. The counting was carried out as for the winter wheats.

Table 3. *Relation of date of drilling of wheat on experimental plots to intensity of August generation gout-fly attack, February* 1945

				Duplicate plots		
No. of plot	Date drilled	Total plants	Mean % attack	Total plants	Mean % attack	S.E. as % of mean
1	25. ix. 44	377	70·0	596	71·5	± 11·4
2	29. ix. 44	499	66·7	629	59·8	± 16·4
3	3. x. 44	424	53·3	354	55·9	± 14·7
4	7. x. 44	316	34·2	359	17·3	± 13·3
5	12. x. 44	320	16·2	287	14·6	± 13·3
6	16. x. 44	393	2·3	378	2·1	± 50·4
7–10	20–31. x. 44	676	0	460	0	0

Table 4. *Relation of date of drilling of wheat in Cornwall to intensity of attack by May generation gout fly, July* 1944

Nos. 1–16 refer to the same fields as in Table 1. The drilling dates for the spring-sown wheats are not known.

Field no.	Total tillers	% attack	Field no.	Total tillers	% attack
	Winter wheats			Winter wheats	
1	402	31·3	13	376	9·0
2	358	30·7	14	362	18·2
3	286	4·8	15	296	35·8
4	234	7·7	16	266	16·5
5	400	27·5		Spring wheats	
6	336	2·9	17	292	91·0
7	278	0·0	18	312	67·3
8	160	2·5	19	258	70·5
9	382	5·2	20	226	75·2
10	380	5·3	21	156	82·1
11	280	30·0	22	230	93·9
12	236	22·5			

Observations in Devon in March 1946

In March 1946 various members of the Department of Plant Pathology, Seale-Hayne College, made observations on the attack of gout fly in Devon on winter-sown wheat. In all, sixty-four fields were examined with a range of date of drilling from 27 September to the first week of December 1945. In most cases the total plants and attacked plants in ten 5 yd. lengths of drill over the field were counted. When extensive tillering had occurred a rough eye estimate of the attack was made.

The results shown in Table 7 indicate that the effect of date of drilling of winter wheat on intensity of the first generation attack is almost identical in Devon and Cornwall.

TABLE 5. *Relation of date of drilling of wheat in Cornwall to intensity of attack by May generation gout fly, July* 1945

Nos. 1–20 refer to the same fields as in Table 2. The drilling dates for the spring-sown wheats are not known.

Field no.	Total tillers	% attack	Field no.	Total tillers	% attack
	Winter wheats			Winter wheats	
1	657	1·2	13	409	0·0
2	398	2·5	14	420	15·7
3	600	1·0	15	370	4·8
4	732	0·7	16	341	2·0
5	720	1·2	17	428	3·2
6	420	2·1	18	524	1·9
7	378	0·0	19	376	0·8
8	436	0·7	20	417	4·7
9	351	0·0		Spring wheats	
10	500	0·0	21	425	48·2
11	278	0·0	22	374	41·9
12	270	0·0	23	380	44·7

TABLE 6. *Relation of date of drilling of wheat on experimental plots to intensity of May generation gout-fly attack, July* 1945

Nos. 1–10 refer to the same plots as in Table 5.

			Duplicate plots	
No. of plot	Total tillers	Mean % attack	Total tillers	Mean % attack
1	322	1·8	420	0·9
2	415	0·3	460	1·5
3	482	1·0	486	1·2
4	300	0·0	752	0·7
5	525	0·2	603	0·7
6	618	0·5	499	3·0
7	429	2·1	444	0·9
8	529	3·8	507	4·7
9	548	2·2	625	2·1
10	429	6·5	543	2·2

TABLE 7. *Relation of intensity of attack by gout fly in March* 1946 *on winter wheat in Devon to date of drilling*

Date drilled	No. of fields	Variation in % attack	Mean % attack
Last week September	3	0–75	50·0
First week October	11	10–90	40·0
Second week October	8	0–90	23·0
Third week October	12	0– 8	1·0
Fourth week October	11	0– 2	0·3
First week November	6	0– 3	0·5
Second week November to first week December	13	0– 0	0·0

EMERGENCE OF FLIES AND NUMBER OF GENERATIONS

May generation

The date of emergence of the generation attacking wheat drilled during the autumn of 1943 was ascertained by collecting a number of damaged plants and placing them in small glass cages with muslin tops. The emergence of adults under caged conditions was checked by frequent field observations. After pupation early in March, more frequent observations were kept; the caging of plants was delayed until the pupae were in an advanced stage in mid-April. Ten attacked plants were collected from each of eight fields and placed in cages. The first flies emerged on 4 May 1944, and emergence continued until 18 May as shown in Table 8.

A similar procedure was followed in 1945, the first fly appearing on 30 April, emergence continuing until 24 May. The results of this emergence are included in Table 8. The first adults, under field conditions, were noted on 13 and 15 May 1944, 1945, respectively.

TABLE 8. *Dates of emergence of adult gout flies from overwintering generation*

	No. of flies emerging			No. of flies emerging	
Date	1944	1945	Date	1944	1945
30 April	0	1	13 May	4	11
1 May	0	0	14	3	14
2	0	1	15	5	4
3	0	0	16	0	0
4	12	0	17	1	6
5	8	0	18	1	3
6	1	0	19	0	0
7	0	7	20	0	0
8	7	7	21	0	0
9	12	11	22	0	3
10	7	8	23	0	1
11	3	19	24	0	2
12	10	15			

August generation

The date of emergence of this generation was recorded, both in 1944 and 1945, as for the previous generation. Material was collected in mid-July and kept in glass cages. About twelve damaged tillers were collected from each of ten fields, using only the ear-bearing portion above the top internode.

Sets were collected from two fields of spring wheat and one field of winter barley in 1944, and from three fields of spring wheat in 1945. The dates of emergence for both years are shown in Table 9.

Number of generations

A general emergence occurred during the second week of May (Table 8). This was similar for both years. A further generation, described in this paper as the August generation, emerged during the first week in August (Table 9). At this time

there was no wheat upon which adults could oviposit, and it was thought there might be a third generation on some intermediate host. In the late summer and autumn of 1944 grasses were examined, particularly couch grass (*Agropyrum repens*), for the presence of eggs. A few eggs, which closely resembled those of gout fly, were found on couch grass in two root fields. None of these few plants could be spared for examination for the presence of larvae. No change was noted until February 1945, when a slight swelling of a few shoots was noted, with the crinkling which is so characteristic of gout-fly attack. In March, unfortunately, the two fields, where the

TABLE 9. *Dates of emergence of adult gout flies from summer generation*

	No. of flies emerging				
	1944			1945	
Date	Winter wheat	Spring wheat	Winter barley	Winter wheat	Spring wheat
27 July	0	0	0	4	0
28	0	0	0	7	0
29	2	0	1	10	0
30	0	0	0	9	0
31	6	0	7	11	0
1 August	10	0	3	15	0
2	14	0	4	23	0
3	11	0	2	29	1
4	12	1	0	17	0
5	11	6	0	16	2
6	9	3	0	16	1
7	11	4	1	8	3
8	2	7	1	1	2
9	3	3	1	5	1
10	1	2	0	3	1
11	0	2	0	2	0
12	0	4	0	1	2
13	0	0	0	1	1
14	0	0	0	0	0
15	1	0	0	0	2
16	0	0	0	0	2
17	0	0	0	0	1
18	0	0	0	0	2
19	0	0	0	0	5
20	0	0	0	0	10

attacked plants were growing, had to be cultivated for spring tillage. The plants were caged but the larvae died before pupation. These observations did, however, support the hypothesis that the cycle commenced by the oviposition in August did not become complete before the following spring.

In the autumn and winter of 1944–5 further information was obtained. Plot 1, sown on 25 September, showed the first shoots above ground on 4 October; the majority of shoots were showing by 9 October, the height of these varying up to 1½ in. The plants of plots 2 and 3 reached this stage on 12 and 16 October respectively. A daily inspection showed the first flies on the very young shoots on 10 October;

the numbers were large by the 12th. Oviposition was, therefore, in full progress on 12 October, and hundreds of eggs were noted varying from one to six per shoot; very few shoots were free from eggs. Oviposition continued freely, with large numbers of flies present, until 18 October; each plot was attacked as soon as shoots were visible. After this date the number of flies decreased rapidly and on the 20th only a few were seen. For several days a few flies could be seen resting on the shoots, but these were less active than those found earlier. The last fly was observed on 9 November. During this period oviposition was noted on wheat under ordinary field conditions.

It was thought unlikely that these flies emerged from an intermediate or third generation considering the investigations made during the late summer of 1944 when only a few eggs were found on couch grass. From observations on hundreds of attacked heads during late August and September, from standing wheat and from stooks it was established that all flies had emerged. Therefore, there was no evidence for a late emergence. It appeared most probable that the adults found in autumn were those that had emerged during August.

The following experiment was carried out in 1945 to investigate this point. A cage $14 \times 14 \times 18$ in. high with a muslin top was erected. Conditions inside the cage were made as nearly natural as possible by adding several species of grass, including couch grass, and dead leaves, the whole being on a 2 in. depth of soil. On 3 August twenty adults (fifteen females and five males) were put into the cage. Flowers were made available each day; honey was also provided. None of the flies was seen to visit the flowers, but they fed readily on the honey. It was difficult to keep an accurate check on the number of flies in the cage, but on 17 August there appeared to be sixteen; two males and two females were added. No pairing was seen during the first week, but ten eggs were found on the couch grass after the first week. By 21 September a few grains of wheat, which had been lying in the stubble, had germinated and eggs were found on the young shoots. The young wheat shoots were removed immediately, as the object of the experiment was to ascertain how long the flies which emerged in August could live.

During September only a few flies could be seen in the cage; when, however, the young wheat shoots were removed the soil was disturbed and several flies emerged from it. The first two pairings were noted on 31 August, with a further one on 4 September. Pairing was general from 21 September. A single grain of wheat had germinated by 5 October; several eggs were laid on the young shoot. Sixteen flies were counted in the cage on 16 October and appeared to be in a healthy condition. By 18 October only three flies remained alive.

A second cage of the same dimensions, containing a pot of wheat, was erected on 3 August; ten flies were introduced. These flies were not fed artificially but were able to live on the juices excreted by the growing wheat until the end of August, but by early September the wheat had died. The majority of the flies died during August, but three remained alive until 18 October with nothing on which to feed.

Several flies collected from the experimental plots in mid-October were compared with those which had been caged from 3 August. These were placed together in a single cage, and it was impossible to divide them into their respective categories. Neither had the appearance of newly emerged flies.

Host preference

General observations on barley (growing alone or with oats) during the summer of 1943 revealed no signs of attack; it was clear that a preference was shown for wheat. Flies bred from the emergence cages previously described were used for further experiments during May 1944. Three additional cages were erected; cage 1 contained wheat, cage 2 barley and cage 3 wheat and barley. On 6 May ten flies were put into each cage. Eggs were observed on 9 May on leaves of wheat in cages 1 and 3. Oviposition continued freely on the wheat for several days. No eggs were observed on the barley in cages 2 or 3. On 15 May one egg was seen on the glass of cage 2 (containing barley only). A wheat plant was added to cage 2 at 10 a.m. on the 16th, and at 2 p.m. on the same day several eggs were observed on the plant; oviposition continued on the wheat as in the other cages.

Experiments could not be carried out with flies from the generation which emerged in August, but they were continued in May 1945. Two cages were used, plants of wheat, barley and couch grass being placed in each. On 10 May twenty flies bred from wheat were added to cage 1, and on 12 May eight flies bred from a field of self-sown barley were added to cage 2. Oviposition took place freely, and on 29 May the plants were examined from each cage and egg counts made. Cage 1 (containing flies from wheat) showed wheat 107 eggs, barley no eggs and couch grass five eggs. The egg count on plants in cage 2 produced the following: wheat forty-nine eggs, barley eleven eggs and couch grass no eggs.

Discussion and conclusions

The investigations reported above confirm that the life history of the gout fly in wheat parallels that reported by Frew for barley. The larvae overwinter in the shoots, pupate in March and give rise to the first generation of flies in May. These oviposit within a few days, the larvae enter the shoots, and pupate in July. The second generation of flies appears in August but does not oviposit until 2 months later. Most of their eggs are laid in the second and third weeks of October.

This delay in oviposition by the second generation has an important bearing on the infection of autumn-sown wheat. Crops not above ground before 20 October escape attack, and it is, therefore, recommended that wheat in the south-west should not be drilled until after 12 October.

Wheat sown before this critical date becomes infected in October and forms the principal source of flies emerging in May. By May the early-sown wheat is sufficiently mature to be unattractive to the flies, which prefer tender growth for oviposition. The young tillers of spring-sown wheat are highly attractive and

consequently severely attacked. Heavy attacks are also frequent in late autumn-sown wheat which develops slowly until the spring, when it makes rapid and tender growth. Wheat drilled in mid-October, however, commonly makes good growth before the winter, so resembles early-sown wheat in being sufficiently mature in May to be unattractive for oviposition.

The fall in percentage attack in the winter of 1944–5 (as compared with that of 1943–4) is probably related to the rainfall for the period of oviposition during both years (Fig. 4). In October 1944 there were periods of torrential rain when large numbers of flies undoubtedly died. Many of the young larvae also perished before they could find refuge within the young shoots. The attack on the plots was more

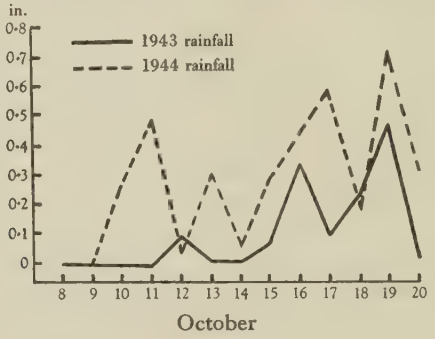

Fig. 4. Rainfall at Falmouth 1943 and 1944, 8–20 October.

intense, as might be expected with only a limited area compared with field conditions; a large number of flies were attracted to the plots before wheat was available elsewhere.

The attack on the plots by the May generation was similar to that in the fields, probably because the plots formed part of the field of wheat. The whole field was drilled on 16 October and plants did not appear above ground before the 26th, when oviposition, for all practical purposes, could be regarded as finished; during May, however, the flies had a much larger area over which to disperse themselves.

The comparatively light attack by the August generation of 1944–5 certainly accounted for the resulting low percentage of the May generation. Oviposition during the second half of May 1945 commenced under favourable conditions, but after the 18th much heavy rain was experienced. Larvae which had emerged were later found to be dead within the leaf-sheath of many tillers. Fig. 5 shows the rainfall for the oviposition period during May 1944 and 1945.

Little has been mentioned about attack by gout fly on barley in the south-west. Emergence dates are given in Table 9 for flies from winter barley. One field of winter barley at Perranporth was very lightly attacked by the May generation in 1944, and it was from this field that breeding material was obtained. A field of

winter barley near Launceston was also lightly attacked; this field adjoined a field of winter wheat with a 30% attack. Both fields of barley were drilled during the first week in October. Flies used for the host-preference experiments were bred from material obtained from a field of self-sown barley near Truro. Oviposition occurred on the self-sown barley between 12 and 20 October, although this was available for several weeks before. Spring barley is only rarely attacked in the south-west, although occasional tillers have been found. Barley is extensively grown and mixed corn containing barley is a frequent crop in Cornwall.

The work suggests the possible existence of a biologic strain of gout fly having a definite preference for wheat.

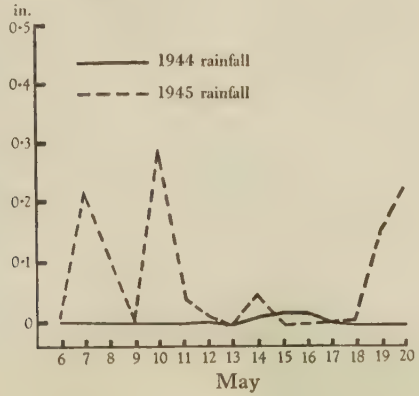

Fig. 5. Rainfall at Falmouth 1944 and 1945, 6–20 May.

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WIREWORMS AND THE SUGAR-BEET CROP: FIELD TRIALS AND OBSERVATIONS

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Observations on wireworms in the sugar-beet crop during 1937–40 indicated the importance of alternative food, such as buried turf, weeds, excess seedlings and interdrilled wheat in determining the degree of injury to the crop.

In 1938, out of thirty-six recorded fields growing sugar beet after ploughed-up grass only one suffered severe wireworm damage. Six of these fields were selected for resowing with sugar beet in 1939, and all suffered moderate or severe attacks. Several other fields in their first year from grass showed only slight damage. These observations were supported by an analysis of the Norfolk War Agricultural Committee crop records for 1940.

Seven trials were carried out in 1939 to determine the effect of increasing the seed rate from normal (about 14–16 lb./acre) to 17–25 lb., and of interdrilling with wheat. In three trials, increasing the seed rate gave significantly higher plant populations before singling. It gave significant increases after singling in one out of two trials. However, in the one trial harvested the increase in 'washed beet' was only 7·6 % and 'total sugar' 15 %. Interdrilling with wheat at 40–70 lb./acre gave significant increases in the plant population before singling in three out of five trials, and after singling in three out of four trials. Increased plant populations both before and after singling were obtained in two other trials with alternating treatments. Increased seed rate and interdrilled wheat together gave greatly improved plant populations before singling in each of two trials and after singling in the one trial where such an observation was possible.

1. Wireworm attack on sugar beet in relation to alternative food

Observations in 1938 and 1939 indicated that when sugar beet is grown on land ploughed up from grass, less wireworm injury occurs in the first than in the second year. This conclusion was apparently confirmed in 1940. The following is a summary of the evidence.

(1) In 1938 thirty-six fields were selected for wireworm experiments or observation plots. The choice generally fell on fields in their first year after grass, as there was every reason for believing that the wireworm population was highest during this first year. Rough sampling confirmed, in most cases, the presence of a sufficiently large population of wireworms to justify the setting out of an experiment. Yet, in all but one of these fields, there was very little wireworm injury even in the control plots. In the exceptional case the wireworm population was nearly 1,000,000 to the acre (samples examined in the field), and the crop was, in fact, severely thinned. However, in the one field in its third year from grass, a relatively small wireworm population produced quite a considerable amount of gapping.

(2) In 1939, with the previous year's experience in mind, fields in their second year from grass were chosen for wireworm experiments. For the most part they

were fields which had been used in 1938. This time they all suffered from wireworm attacks. In addition, a few fields in their first year after grass were kept under observation and it was found that they did not suffer greatly from wireworm attack.

(3) In 1940, 1500 acres of newly ploughed grassland were sown with sugar beet in the county of Norfolk. Analysis of reports from district officers gave the following percentages of acreages in the different categories:

Failure	Poor	Fair	Good	Very good
3	7	42	40	8

This shows that some 90% of the acreage sown could be regarded as successful despite the variety of factors (wireworms and others) which might have affected the crops.

(4) There is a widespread belief among East Anglian farmers that damage is worse in the second than in the first year.

Accepting the fact of relative immunity in the first year, there can be little doubt that one important contributory factor is the presence of buried turf. Thus, for example, in 1938 when a trial was conducted in a beet crop following upon grass, numbers of wireworms could be found feeding on the buried turf, but there was no damage to the beet and very little to the wheat drilled between the beet. This was the most striking example, but similar observations were made in other cases.

In the exceptional case quoted above when a serious wireworm attack occurred in the first year, a very considerable portion of the turf had been harrowed into heaps and burnt. Precisely how much turf was removed in this manner is not clear, but it must certainly have resulted in a great reduction in the amount of food available for the wireworms.

It seems probable from these and other observations that the extent of wireworm feeding upon the beet crop is dependent upon the wireworm population and the amount of alternative food. Thus, when the wireworm population is extremely high, an attack may be expected, even when the whole of the turf is ploughed in. Conversely, if the turf is largely removed by cleaning operations, even a small wireworm population may have quite a serious effect on the crop. Again, any alternative food would tend to reduce the extent of the damage. For example, interdrilling with wheat might be expected to have this effect. When a high seed rate has been employed the excess of seedlings, up to the time of singling, may be regarded as alternative food. Again, cases are known where a beet crop has escaped serious damage apparently because of the foul condition of the land, the weeds serving to attract a proportion of the wireworms. In one case a grower deliberately followed the maxim: 'Half a crop on foul land is better than no crop on clean land.'

2. Assembly of wireworms in wheat and beet rows

In a trial carried out in 1940 and designed to test the effect of interdrilling with wheat, an attempt was made to assess the number of wireworms assembled in the wheat and beet rows. The investigation was limited to a strip running at right angles

across the trial plots and covering all treatments and replications. The strip was chosen in a reasonably uniform area where wireworm damage appeared early. In each plot within the strip, shortly after the beet appeared above ground, three random samples of 1 foot of row were examined and the number of wireworms present counted. On the wheat-treated plots, the number of wireworms present in the adjacent foot lengths of wheat row were also counted. The mean numbers of wireworms per foot of row on the various plots were analysed statistically on the basis of the experimental lay-out to see whether the wheat treatment had affected the assembly of wireworms. The results are summarized in §§ (1) and (2) of Table 1, while § (3) of the same table gives the comparison of the wheat and beet rows of the treated plots to which the *t*-test only is applicable.

TABLE 1. *Assembly of wireworms in the beet and wheat rows. Wireworms per foot of row*

(1) In the beet rows of the wheat-treated plots	2·48	In the beet rows of the untreated plots	4·16	S.E. 0·8	Difference insignificant
(2) Total in the wheat and beet rows of the treated plots	12·16	In the beet rows of the untreated plots	4·16	S.E. 1·1	Difference significant
(3) In the wheat rows of the treated plots	9·68	In the beet rows of the treated plots	2·48	$t=10·5$ ($n=16$)	Difference significant

The total number of wireworms assembled (wheat and beet rows together) in the wheat-treated plots was greater than the number in the untreated plots (beet rows only), and, within the treated plots, the wheat rows contained considerably more wireworms than the beet rows. In all the trials it was noted that the wheat germinated before the beet and provided many more seedlings per unit length of row. The protective action of the wheat seems to be due to the assembly of a considerable proportion of the wireworms in the wheat rows at an early stage. Greater advantage might be gained if the wheat were drilled some days in advance of the sugar beet to obtain maximum assembly in the wheat rows.

3. FIELD TRIALS

Investigations by Miles & Petherbridge (1927) into the use of baits for the control of wireworms showed that wheat and oats acted as attractants even when drilled in growing crops. Petherbridge (1938) described a trial in which the method was applied to the sugar-beet crop on wireworm-infested land; an increased seed rate was also tried. The striking results obtained in this trial led to more extensive tests by the present authors in 1938. Thirty-six fields ploughed up from grass and destined for sugar beet were sampled for wireworms and thirteen were considered suitable for full-scale trials. Unfortunately, in all but one field, there was insufficient wireworm damage to give any comparison between the treated and untreated plots. The experiments were repeated on six of these fields and one other field in 1939, and severe wireworm damage occurred in all seven fields.

In all the trial fields a preliminary sampling was carried out during the early spring before drilling began. From ten to twenty samples, each 6 in. square and 12 in. deep, were examined in the field. The resulting estimates of wireworm populations are considerably lower (half or less) than would be obtained by a washing method.

The drilling was carried out by the grower under supervision. The only modification of the usual drilling practice was a higher seed rate and the drilling of wheat between the beet rows in certain of the plots. Practical details varied from trial to trial according to the type of drill available. In trials 2, 3 and 4 each wheat-treated plot had three rows of beet with wheat on both sides, but in trials 1, 6 and 7 each wheat-treated plot had only two rows of beet with wheat on both sides. In trial 5 only the middle row had wheat on both sides, but by combining two drill-widths in one plot two such rows were made available. In trials 1, 2, 3, 4, 6 and 7 the beet was drilled first and the wheat either immediately afterwards or the following day at the latest; in trial 5 the beet and wheat were drilled simultaneously. Randomized block lay-outs were employed in five trials, and simple alternation of treated and untreated plots in two others.

In all trials an estimate of the plant population was made before singling. The number of beet seedlings in a 12 in. sample of row was noted, but wireworm-damaged seedlings were not counted. In trial 6, where the first estimate was made, forty random samples were taken in each plot, but in subsequent estimations the number of counts was increased to forty-eight. The samples were distributed so that an equal number was taken in each quarter of the plot. In trial 4, three rows out of the four in each plot were employed for the purposes of the estimation. It was discovered, however, that more satisfactory comparisons could be made if observations were restricted to the two inner rows of each set of four (where a four-coulter drill had been used) or the middle row of each set of three (where a three-coulter drill had been used). By this means the same coulters were compared and therefore differences due to coulters were eliminated.

From the forty or forty-eight counts in each plot, a figure representing the average density of the plant population within the plot was calculated. This figure offers a basis for the comparison of different plots, but it is not of absolute value for the comparison of treatments. The yield or 'total sugar' is the ultimate criterion. The data relating to the initial plant population are of interest, nevertheless, as experience has shown that a fairly high population is necessary if a reasonably full plant is to be obtained after singling.

Some time after singling a count was made of the total plants in each plot (the same rows being considered). This figure is much more nearly related to gross yield but is still only an approximate estimate of it, owing to the compensation around gaps. However, where yields cannot be ascertained, it is a reasonably satisfactory basis of comparison between treatments.

Owing to the outbreak of war in September 1939, arrangements for the separate

lifting and loading of roots from individual plots had to be abandoned. In three of the trials, however, it was found possible to keep the roots from plots of the same treatment together and thus to obtain a comparison of treatment totals but sacrificing all the benefits of replication. The yields and sugar contents were assessed at the British Sugar Corporation beet factory at Stoke Ferry, King's Lynn.

Trial 1. The land had been derelict for several years. It was ploughed in 1938 and put down to beet. A field estimate in the spring of 1938 gave 900,000 wireworms per acre; in the spring of 1939, about 700,000. It was drilled on 8 May at the rate of about 14 lb./acre. The treatments were: (1) no wheat, (2) wheat at 42 lb./acre, (3) wheat at 70 lb./acre. The plots ($\frac{1}{13}$ acre, excluding discards) were randomized with nine replications.

Up to the second week in June, the treated plots were obviously better than the controls, but thereafter comparisons became more difficult owing to weeds. The wheat was seriously thinned by birds and after the first 3 weeks there was very little left. The wireworm attack was very patchy, some areas being almost denuded of beet, whereas others had a good plant.

TABLE 2. *Trial* 1*: effect of wheat baiting on plant populations and yield*

	Treatments				
	No wheat	Wheat 42 lb./acre	Wheat 70 lb./acre	Mean	S.E.
Plant populations					
Before singling					
Plants per foot	4·34	6·74	7·54	6·21	0·53
% of control	100	155·2	173·6		
After singling					
Plants per acre	10,055	13,149	12,456	11,787	584
% of control	100	130·8	123·9		
% of full plant	38·5	50·3	47·7		
Yield					
Washed beet (tons/acre)	6·54	8·19	7·53	7·42	*
Sugar content (%)	14·4	14·0	14·7	14·4	*
Total sugar (cwt./acre)	18·8	22·9	22·1	21·4	*

* No data available for calculating S.E.

The treatments as a whole produced highly significant differences in the plant populations before and after singling (see Table 2). There were no significant differences, however, between the effects of the two wheat rates. Both applications of wheat gave increased yields of both washed beet and total sugar, but the actual yields were well below the average for this type of land.

Trial 2. The field had been down to grass for an unknown period. It was ploughed in 1938, the turf being buried deeply, and was drilled with beet. The wireworm population was estimated at 350,000 per acre both in 1938 and 1939, but the 1938 crop was strikingly free from damage. Beet was drilled on 4 May and wheat on 5 May. The following treatments were applied: (1) no wheat, (2) wheat at

40 lb./acre, (3) wheat at 70 lb./acre. The plots ($\frac{1}{20}$ acre) were randomized with eight replications.

The wireworm attack was rather more uniform than in trial 1. Differences between treated and untreated plots were visible at an early stage and continued until well after singling. Rodents and, to a lesser extent, birds attacked the wheat so that it soon became rather thin.

TABLE 3. *Trial 2: effect of wheat baiting on plant populations*

	Treatments				
	No wheat	Wheat 40 lb./acre	Wheat 70 lb./acre	Mean	S.E.
Plant populations					
Before singling					
Plants per foot	7·72	9·87	11·08	9·56	0·186
% of control	100	127·9	143·4		
After singling					
Plants per acre	27,629	29,014	30,820	29,149	200·6
% of control	100	105·0	111·5		
% of full plant	83·2	87·4	92·9		

The treatment effects, as a whole, were highly significant (see Table 3). Wheat at the higher rate produced a significantly higher plant population than wheat at the lower rate, both before and after singling; the linear component of the regression was insignificant. No yield data were obtained.

Trial 3. The field had been down to grass for many years before being ploughed up in 1936. Various crops from 1936 to 1938 were reported to have been badly attacked by wireworms. The wireworm population in the spring of 1939 was

TABLE 4. *Trial 3: effect of heavy seeding and wheat baiting on plant populations and yield*

	Treatments					
	Normal seed rate (15 lb./acre)		Heavy seed rate (25 lb./acre)			
	No wheat	Wheat 60 lb./acre	No wheat	Wheat 60 lb./acre	Mean	S.E.
Plant populations						
Before singling						
Plants per foot	6·71	8·29	9·70	14·25	9·75	1·50
% of control	100	123·6	144·5	213·1		
After singling						
Plants per acre	18,775	21,047	19,792	23,422	20,759	798
% of control	100	112·1	105·4	124·7		
% of full plant	64·7	72·6	68·3	80·7		
Yield						
Washed beet (tons/acre)	9·27	9·37	9·70	10·35	9·67	*
Sugar content (%)	14·4	14·9	15·4	15·9	15·15	*
Total sugar (cwt./acre)	26·7	27·9	29·9	32·9	29·3	*

* No data available for calculating S.E.

estimated at 500,000 per acre. The field was drilled on 18 April and the following treatments applied: two seed rates, viz. 15 and 25 lb./acre, no wheat, and wheat at 60 lb./acre. The treatments were used in all four combinations. The plots ($\frac{1}{30}$ acre) were randomized and replicated six times.

Soon after germination obvious differences appeared between the treated and untreated plots. Those with a high seed rate were the best, but the wheat plots were also clearly better than the controls. Slight attacks of pigmy beetle and blackleg reduced the plant population to a certain extent but not enough to interfere with the experiment.

The effect of the higher seed rate on the plant population before singling was highly significant, but the effect on the plant population after singling fell just short of significance (see Table 4). The effect of the wheat, on the other hand, was insignificant before singling but highly significant after singling. The effect of combined wheat and high seed-rate treatment was very pronounced, but the interaction between the two types of treatment proved, rather unexpectedly, to be insignificant. Yields of washed beet and total sugar appear to have been increased by the treatments, especially when combined.

Trial 4. The field had been down to grass for 16 years. It was ploughed in the spring of 1938 and drilled with beet. Little wireworm injury was experienced except in one or two patches, although the population, as estimated in the field, was about 300,000 per acre. In 1939 the population was re-estimated at 450,000. Drilling was carried out on 20–21 April when the following treatments were applied: normal seed rate (14 lb./acre) and high seed rate (17 lb./acre) in all four combinations with wheat and no-wheat. The plots ($\frac{1}{20}$ acre) were randomized and replicated six times.

Soon after germination the seedling beet was extensively attacked by wireworms. The plant was seriously thinned in certain parts of the experimental area. There was considerable gapping in the treated plots, but the untreated plots were, on the whole, very much worse. The grower eventually decided to redrill the field although this meant losing the benefit of the moderate plant on the treated plots.

TABLE 5. *Trial 4: effect of heavy seeding and wheat baiting on plant population before singling*

	Treatments					
	Normal seed rate (14 lb./acre)		Heavy seed rate (17 lb./acre)			
	No wheat	Wheat 50 lb./acre	No wheat	Wheat 50 lb./acre	Mean	S.E.
Plant population before singling						
Plants per foot	3·55	6·50	5·34	7·24	5·66	0·49
% of control	100	183·4	150·6	204·2	—	—

The treatments produced significant differences, that due to interdrilling with

wheat being highly significant (see Table 5). Increased seed rate and wheat together produced a highly significant increase, but the interaction of the two treatments was insignificant.

Trial 5. The field had been derelict for several years. A wheat crop in 1938 was much damaged by wireworms but recovered later. In the spring of 1939 the wireworm population was estimated at about 600,000 per acre. Drilling was carried out on 25 April, the following treatments being applied: normal seeding (16 lb./acre) and heavy seeding (22 lb./acre) with wheat and no-wheat subplots (wheat at 40–50 lb./acre). The whole-plots (seed rates) were replicated five times. Because of certain unsatisfactory features of the drilling, the data were analysed on the basis of two separate trials.

The early plant was rather irregular owing to mole damage. There was a moderate amount of wireworm damage, but little difference could be seen between the treated and untreated plots. There was, however, an abundance of wireworms feeding on the wheat. A considerable proportion of the latter was removed by birds before the beet reached the singling stage. After singling, only slight differences between the treatment effects could be observed.

TABLE 6. *Trial 5: effect of (a) wheat baiting, (b) seed rate on plant populations*

	(*a*) Treatments			
	No wheat	Wheat 45 lb./acre	Mean	S.E.
Plant populations				
Before singling				
Plants per foot	11·8	13·5	12·7	0·60
% of control	100	114·2	—	—
After singling				
Plants per acre	18,177	18,663	18,420	355
% of control	100	102·8	—	—
% of full plant	57·9	59·4	—	—

	(*b*) Treatments			
	Normal 16 lb./acre	Heavy 22 lb./acre	Mean	S.E.
Plant populations				
Before singling				
Plants per foot	8·5	14·4	11·4	0·27
% of control	100	169·5	—	—
After singling				
Plants per acre	16,290	19,195	17,743	385
% of control	100	117·8	—	—
% of full plant	51·9	61·1	—	—

The higher seed rate had a significant effect on the plant population both before and after singling (see Table 6). The wheat effect, however, fell short of the 5% level of significance before singling and was quite insignificant after singling. Its comparative failure may be ascribable to the early removal of the wheat by birds.

Trial 6. The field had been derelict for some years. It was ploughed and cropped with sugar beet in 1938. The crop was not much affected by wireworms, but rooks in search of the latter caused much thinning by pulling up the plants. In the spring of 1939 the wireworm population was roughly estimated at almost a million to the acre. Beet was drilled on 24 April and the following treatments applied: half the field was drilled at 16 lb./acre and the other half at 20 lb./acre; within each half there were six plots with wheat interdrilled at 36 lb./acre alternating with six untreated plots.

TABLE 7. *Trial* 6*: effect of wheat baiting at normal and heavy rates of (beet) seeding on plant populations and yield*

	Treatments			
	Normal seed rate (16 lb./acre)		Heavy seed rate (20 lb./acre)	
	No wheat	Wheat 36 lb./acre	No wheat	Wheat 36 lb./acre
Plant populations				
Before singling				
Plants per foot	6·50	8·45	8·97	11·12
% of control	100	129·9	100	124·0
After singling				
Plants per acre	20,263	23,163	21,219	23,073
% of control	100	114·3	100	108·7
% of full plant	69·6	79·6	73·1	79·5
Yield				
Washed beet (tons/acre)	8·95	8·81	7·56	8·34
Sugar content (%)	14·2	14·2	14·2	14·0
Total sugar (cwt./acre)	25·4	25·0	21·5	23·4

Note. Seed-rate treatments not replicated; wheat treatments replicated but not randomized.

A fairly good plant was obtained in the early stages, but gradually the control plots deteriorated and were soon obviously worse than the treated plots. In both halves of the field, the wheat-treated plots showed higher plant populations, both before and after singling (see Table 7). The yield data, however, suggest that wheat depressed the yield very slightly at the normal rate of seeding but increased it at the higher rate of seeding.

Trial 7. The field had been down to grass in a semi-derelict condition for many years. The turf was ploughed in deeply in the spring of 1939. The wireworm population was estimated at 800,000 per acre.

The initial plant was fairly good, but later it was somewhat thinned in patches. The crop became foul with couch grass at an early stage, and cleaning operations resulted in a further reduction in the plant.

The wheat-treated plots had slightly higher plant populations than the untreated plots (see Table 8).

TABLE 8. *Trial 7: effect of wheat baiting on plant populations*

	Treatments	
	No wheat	Wheat (40–50 lb./acre)
Plant populations		
Before singling		
Plants per foot	12·18	13·60
% of control	100	111·7
After singling		
Plants per acre	20,202	21,116
% of control	100	104·5
% of full plant	69·6	72·6

4. DISCUSSION AND CONCLUSIONS

The treatment effects recorded in the different trials are summarized in Table 9.

TABLE 9. *Summary of treatment effects in all seven trials*

			% increase and its significance			
			Plant populations		Yield	
Trial	Treatments and replications	Treatment comparison, interaction, regression	Before singling	After singling	Washed beet	Total sugar
1	O W_1 W_2 ×9	W_1 *v.* O	55·2**	30·8**	25·3	21·8
		W_2 *v.* O	73·6**	23·9*	15·2	17·6
		W_2 *v.* W_1	11·8⁰	− 5·3⁰	− 8·1	− 3·5
		Regression	⁰	⁰	—	—
2	O W_1 W_2 ×8	W_1 *v.* O	27·9**	5·0*	—	—
		W_2 *v.* O	43·4***	11·5**	—	—
		W_2 *v.* W_1	12·1*	6·2**	—	—
		Regression	⁰	⁰	—	—
3	NO NW HO HW ×6	H *v.* N	60·0**	8·5⁰	7·6	15·0
		W *v.* O	37·7⁰	15·3**	4·0	7·4
		Interaction	⁰	⁰	—	—
		HW *v.* NO	113·1*	24·7***	11·2	23·2
4	NO NW HO HW ×5	H *v.* N	25·2*	—	—	—
		W *v.* O	54·7**	—	—	—
		Interaction	⁰	—	—	—
		HW *v.* NO	104·2***	—	—	—
5(*a*)	O W ×9	W *v.* O	14·2⁰	2·8⁰	—	—
(*b*)	N H ×4	H *v.* N	69·5*	17·8*	—	—
6	(at N) O W	W *v.* O	29·9	14·3	− 1·6	− 1·6
	(at H) O W	W *v.* O	24·0	8·7	10·3	8·8
7	O W	W *v.* O	11·7	4·5	—	—

Key to Symbols

Treatments		Significance levels	
Normal seed rate	N	0·1 %	***
Heavy seed rate	H	1 %	**
Without wheat	O	5 %	*
With wheat	W	Insignificant	⁰
Light application	W_1	Significance not known	No symbol
Heavy application	W_2		

The plant population before singling appeared to be very responsive to both the heavy seeding and wheat treatments. The response to increased seed rate was significant in all three randomized block trials in which it was applied, though in two of them the level of significance was rather low. The wheat treatment produced a significant response in three out of five trials. Although in one case a heavy application of wheat produced a significantly greater response than a light application, there was on the whole no indication of a significant linear response to increasing rates of application of wheat.

In comparing the seed rate and wheat effects before singling, allowance must be made for the fact that the times at which the plant counts were made in the different trials were not strictly comparable. The counts in some of the trials were made nearer the time of singling than they were in others. It will be realized that a count made immediately after germination (say before the wireworms had had time to produce an appreciable effect upon the plant population) would reveal a relationship between the heavy seed-rate plots and normal seed-rate plots almost exactly equal to the ratio of the seed rates; on the other hand, wheat could have had little effect at this stage. The later the count is made, therefore, the more marked should be the effect of the wheat.

The population after singling revealed a much less marked response to treatment. This, in fact, is only what may be expected when it is realized that the process of singling results in the removal of more plants from a thick stand than from a thin stand, and therefore has a levelling effect. Increased seed rate produced a significant response in three cases but not in the fourth. As in the plant population before singling, so in the population after singling, the response to increased wheat rate is not of a simple linear type. Within the limits of the treatments applied in these trials, there is no interaction between seed rate and presence or absence of wheat. In other words, the application of one treatment does not reduce (or increase) the benefit derived from the application of the other treatment.

Yields were obtained for only three of the trials and the information applied only to treatment totals. The yields, in all cases, were below normal for the type of land on which the crops were grown. Heavy seeding apparently increased the yield of washed beet and total sugar in the one trial for which the information was available. Wheat baiting increased the yield in two trials. In the third (trial 6) the results were conflicting, but the average effect was a small increase.

One of the difficulties of the method of interdrilling with wheat is the destruction of the wheat plant by birds. This difficulty was experienced on all but one trial and, in the exceptional case, rodents did at least a part of what the birds had left undone. It appears that the birds were mainly seeking wireworms though they may also have been feeding on the wheat grain. By removing the wireworms they were, of course, contributing to a reduction of damage to the beet, but by destroying the wheat they were liberating wireworms to feed on the beet. During plot inspections it was frequently observed that the beet plant was thinnest where the wheat had been

thinned by birds, but it would be unjustifiable to infer from this that the beet had been thinned because there was insufficient wheat in these areas. The net effect of the destruction of interdrilled wheat by birds is not known, but, in addition to pulling up the wheat, birds frequently uproot beet seedlings, even after the singling stage. Many growers believe that the wheat is largely responsible for the activities of birds in a beet crop interdrilled with wheat. While this may be a disadvantage attached to the use of wheat, it does not affect the validity of the results given above, as these results measure the sum of all effects associated with wheat.

An indication of the practical value of the wheat-baiting and heavy seeding in reducing the severity of wireworm attack may be obtained from the data for *Percentage of full plant* in Tables 2–8. The theoretical full plant has been calculated from the known spacings (row widths and chopping-out distances) for each trial. In general, although both treatments give higher plant populations, these are still appreciably lower than could be desired. Naturally, even on crops unaffected by wireworm a full plant is never achieved and very rarely approached. A better indication is given by the yield data, where available. Even the best treatment totals are lower than could be expected from such soils in the absence of wireworms. It is concluded therefore that wheat-baiting and heavy seeding may, in certain circumstances, improve plant establishment but cannot be relied upon to produce a full plant. The conditions under which these measures are most effective are imperfectly understood, but, in all probability, one of the important factors is the existence of a moderate wireworm population—possibly about 1,000,000 per acre (washing method).

Heavy seeding has long been recommended for sugar beet as a precaution against thinning by wireworms or other pests, but wheat-baiting has not, until recently, been used other than experimentally. However, several growers used the method in 1939 and 1940, but only two cases were investigated by the present authors. In one of them the grower proposed to drill wheat on the whole field but agreed to leave three separate drill-widths untreated for comparison with the rest of the field. A remarkable degree of control was obtained, as shown by the very much thinner plant on the untreated parts. In the other case investigated, the grower obtained a satisfactory plant establishment on a block of three fields which had persistently suffered from wireworm attack when sown to beet. In this case, however, it was not certain that the improvement was wholly due to the use of wheat. Other instances of successful use of the wheat-baiting method have been reported. Owing to the need for conserving cereals, the wheat-baiting method was not officially recommended after 1940.

The practical advantages of an efficient soil insecticide for the control of wireworms in the sugar-beet crop is indicated indirectly by the data in Table 9. It will be noted that the improvement in the initial plant population is not always reflected in the population after singling, nor is the latter necessarily closely correlated with the yield. Such divergencies are attributable to at least two important factors. In the

first place, heavy seeding increases the initial plant population over the field as a whole, but wireworm damage is usually patchy in distribution. Consequently, some areas may be left with great excess of seedlings needing early singling, while others will have a suboptimal number of seedlings which would benefit from late singling. The actual time of singling is inevitably a compromise, with the result that a depression of yield of varying magnitude occurs outside and within the patches. Wheat is less harmful in this respect because it can affect the stand only in the presence of wireworms. After singling the increased seed rate can have no further effect in protecting the plants. Wheat, however, can continue to do so as long as it is allowed to remain, but in practice it does not remain long after singling. Consequently the singled plants receive little or no protection at a time when they are still vulnerable to wireworm attack. Although a high proportion of singled plants may survive they may be so crippled as to be unable to contribute fully to the usual compensation around gaps. An efficient soil insecticide (such as benzene hexachloride appears to be) allows the crop to approach the singling stage more uniformly and, in addition, provides such protection as may be required for a short time after singling. It matters little, as far as the beet crop is concerned, whether the protection during the vulnerable period is provided by the killing of the wireworms or by their temporary inactivation; the half-grown crop is comparatively resistant to attack.

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THE TOXICITIES OF THREE PETROLEUM OILS TO THE GRAIN WEEVILS

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(With 4 Text-figures)

The toxicities of three petroleum oils to *Calandra granaria* L. and *C. oryzae* L. have been determined. The oils were Shell oil P31, Odourless Distillate (O.D.), and Pool burning oil (P.B.O.). At 20 and 25° C. *C. oryzae* was more resistant than *C. granaria* to a direct spray of P31. At 20° C. *C. oryzae* was more resistant than *C. granaria* to direct sprays of O.D. and P.B.O. The relative toxicities to both species of direct sprays of the three oils could be expressed as: P.B.O. $<$ P31 $\geqslant$ O.D. $\geqslant$ P.B.O. *C. oryzae* was the species more resistant to films of P31 on Whatman no. 544 filter-paper.

Films of P31 or a P31/water emulsion on brick, and films of P31 on sacking and cement, were non-toxic to *C. granaria*, but films of P31 on cement pretreated with gelatin were highly toxic to this species.

Beetles that received doses of P31 a little in excess of those sufficient to knock them down rarely recovered completely. P31 probably suffocates the beetles by blocking their spiracles and/or tracheae.

P31 appears to be the most useful of the three oils for the control of *C. granaria*. In practice it should be effective as a direct spray, though not as a film, and could therefore be used to control infestations in which beetles were exposed. Films of P31 on surfaces suitably pretreated might, however, be used for control.

Introduction

Solutions of pyrethrins in Shell oil P31 have been widely used as sprays for the control of stored product insects, but during the war, owing to a shortage of pyrethrum, substitutes were sought. As it was known from laboratory experience that granary weevils, adult *Calandra granaria*, were much more susceptible to P31 than other beetles such as *Tribolium castaneum*, it appeared that the former species might be controllable by P31 alone. Preliminary laboratory experiments indicated that a direct spray of 1·5 mg./sq.cm. of P31 should kill 95% or more of *C. granaria*, though films of P31 would be of little or no use for control. On this basis a recommendation for the control of residual populations of *C. granaria* in empty stowages by a heavy direct spray of P31 was made to the Ministry of Food, Infestation Division. The Ministry, however, shortly afterwards wished to control heavy infestations of rice weevils, adult *C. oryzae*, and tried a direct spray of P31 for the purpose, with disappointing results. This suggested that *C. oryzae* might be more resistant than *C. granaria* to P31, and the relative resistance of the two species to P31 was therefore determined. Recovery of beetles from P31 poisoning was investigated, and further experiments were carried out on the toxicity of P31 films.

In addition, the relative toxicities of P31, Odourless Distillate, and Pool burning oil were investigated.

Since the war ended, it was discovered that during and before the war oil/water emulsions were used extensively in Germany for the control of *C. granaria* (see Parkin & Oxley, 1945). No toxicity data for these emulsions are available.

Specifications of the oils

The three petroleum oils, Shell oil P31, Odourless Distillate (O.D.), and Pool burning oil (P.B.O.), are respectively a highly refined oil of the medicinal paraffin type, a highly refined kerosene, and a cruder kerosene. The specifications of P31 and O.D. below are taken from Robinson (1942). The characteristics of P.B.O. vary considerably, so that the figures for this oil are approximate.

	P31	O.D.	P.B.O.
Specific gravity (15–16° C.)	0·857	0·779	0·805
Initial boiling-point (° C.)	300	198	150
Final boiling-point (° C.)	—	257	280
Distillation at 200° C. (%)	—	—	30
Viscosity Redwood I at 20° C. (sec.)	141	32	—
Flashpoint open (° C.)	169	—	—
Flashpoint closed (° C.)	160	71	45
Unsulphonatable residue (%)	99·2	99	—
Colour Saybolt	—	—	20

Experimental technique

The test insects were *Calandra oryzae* L. of the large form (see Richards, 1944; Birch, 1944), and *C. granaria* L. The beetles were taken from insectary cultures, and were of unknown age. They were counted into batches of fifty, and conditioned for about 16 hr. at 25° C. and 70% R.H. before being sprayed or exposed on films.

The beetles were sprayed by means of the direct-spray technique described by Hewlett (1947). The spraying apparatus, a modified form of the tower designed by Potter (1941), was fitted with atomizing nozzle P.I.L. no. AN1, adjusted to a setting of $\frac{5}{8}$ turn (see Hewlett, 1946). The V/D ratios (see Hewlett, 1947) for O.D. and P.B.O. were nearly equal to that for P31, but were more variable. To prevent the beetles from escaping while they were sprayed, it was necessary to smear lightly with P31 the duralumin ring enclosing them. Tests showed that the P31 on the ring exerted no toxic effect on the beetles.

In film tests P31 and a P31/water emulsion only were used, because P31 is the only oil of the three sufficiently non-volatile to form persistent films. The emulsion was of the following composition:

P31 + 1% cholesterol	1 vol.
Water + Teepol X	1 vol.

The samples of brick, cement and sacking were similar to those employed by Parkin & Hewlett (1946). Some cement samples were pretreated with 5% w/v gelatin as by Hewlett & Parkin (1947).

Whatman no. 544 filter-papers were sprayed as in the film technique of Parkin & Green (1943). Alternatively, P31 was applied as evenly as possible to Whatman no. 1 filter-papers 7 cm. in diam. by means of a capillary pipette, and the oil was allowed to spread for 30 min. before insects were confined on the papers. The samples of cement and sacking were sprayed, and the deposit on them estimated, as described by Parkin & Hewlett (1946). Samples of brick, because of the heavy deposits (up to 15 mg./sq.cm.) applied, were sprayed by means of a B.E.N. spray gun S1, the deposits being estimated roughly by means of weighed cover-slips.

Insects were confined on the films within glass rings 6 cm. in diameter and 2 cm. in height. The rings were fixed to the surfaces of cement and brick samples by external seals of plasticine.

In assessing the toxic effects of the oils the insects were examined under a low-power binocular microscope. An insect was considered dead if it neither moved spontaneously nor responded by reflex movement on slight pressure with a soft brush. An insect was counted as knocked down if it lay on its back, unable to regain the normal position; knock-down in this sense included death.

Results

Symptoms of oil poisoning

In batches of insects exposed to films of P31 on Whatman no. 544 filter-paper, the percentage of beetles knocked down increased over a period of days. In the individual insects, however, the development of symptoms of oil poisoning occupied up to about $\frac{1}{2}$ hr. A beetle began to walk slowly, and, in contrast, for instance, with pyrethrum poisoning, it was neither stimulated nor suffered marked loss of co-ordination. The beetle stopped walking, its legs flexed beneath it, and it fell over on to its back. The majority of affected beetles were immobilized completely, and the remainder moved only slightly.

Insects that succumbed to a direct spray of P31 were incapacitated about 4–6 min. after being sprayed, and the development of poisoning was similar to, though more rapid than, that due to P31 films.

Knock-down by a direct spray of O.D. or P.B.O. took place less than 4 min. after spraying, and its onset was even more rapid than that due to a direct spray of P31. Complete recovery from poisoning by knock-down by O.D. or P.B.O. was more frequent than that by P31, and recovery from poisoning by the first two oils followed a characteristic course. As a beetle recovered its legs began to move feebly, and the coxal and thoracico-cephalic movements were regained. The beetle then began to walk in a peculiar halting manner, with tibiae and tarsi abnormally flexed, and without movement at the femoro-tibial or tibio-tarsal joints. The legs eventually recovered, but in no particular order, though in each leg the femoro-tibial movement usually returned before the tibio-tarsal. A single leg sometimes lacked both movements when all the other legs had recovered fully.

Direct-spray tests

The resistances of the two species to a direct spray of P31 were compared. The beetles, in batches of fifty, were sprayed at 20° C. and 70% R.H. and afterwards kept at 20 or 25° C. and 70% R.H. The results of the tests are shown graphically in Fig. 1, in which each point represents the mortality in one batch of insects 24 hr. after spraying. The control mortality was nil. The data were homogeneous, and the lines have been fitted by eye. Fig. 1 shows that *C. oryzae* was definitely more resistant to P31 than *C. granaria*, whether the insects were kept at 20 or 25° C. after spraying. The L.D. 50's and the slopes of the regression lines were as follows:

		C. granaria	*C. oryzae*
L.D. 50 (mg./sq.cm.)	20° C.	0·955	1·85
	25° C.	0·908	1·96
Slope	20° C.	10·7	8·4
	25° C.	15·0	9·5

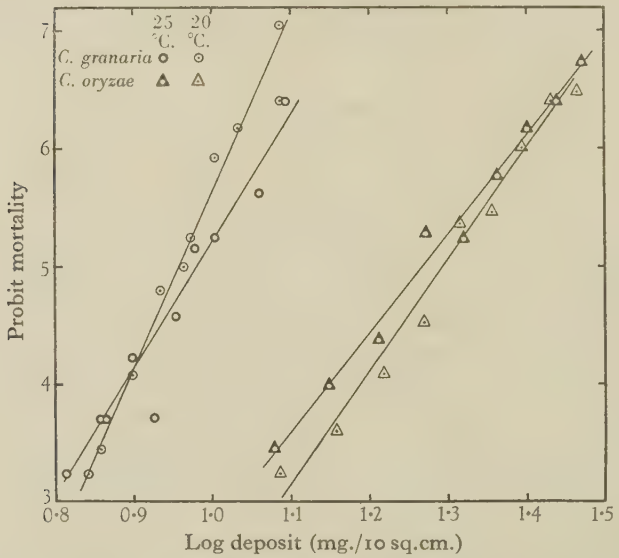

Fig. 1. The toxicity to *Calandra* spp. of a direct spray of P31 when the beetles were kept at 20 and 25° C. after being sprayed.

The regression lines for *C. granaria* both have a steeper slope than those for *C. oryzae*, while the lines for 25° C. were both steeper than those corresponding for 20° C. The effect of temperature after spraying on the L.D. 50's was unimportant.

The toxicities of P31, O.D. and P.B.O. to both species were determined in two separate experiments, using one species in each. The insects were sprayed at 20° C. and 70% R.H., and kept under the same conditions afterwards. Tables 1 and 2 give the respective results of the two experiments, showing the knock-down and mortality observed in batches of fifty insects 24 hr. and 3 days after spraying.

TABLE 1. *The toxicity to* Calandra granaria *of a direct spray of* P31, *Odourless Distillate, and Pool burning oil at* 20° C.

Oil	Deposit (mg./sq.cm.)	'Mortality' (%) 24 hr.	'Mortality' (%) 3 days	Knock-down (%) 24 hr.	Knock-down (%) 3 days
P31	0·67	8	6	10	6
	0·80	44	40	44	40
	0·90	70	66	72	66
	1·00	94	100	100	100
	1·11	96	96	96	96
	1·20	96	96	96	98
	1·32	100	100	100	100
O.D.	0·53	2	0	2	0
	0·76	20	14	22	14
	1·06	82	58	94	92
	1·36	98	60	98	98
	1·75	90	52	96	92
	2·05	96	50	100	100
	2·28	96	74	100	100
P.B.O.	0·51	2	0	2	0
	0·73	10	2	10	4
	1·02	48	28	52	34
	1·32	58	46	74	56
	1·68	92	62	100	98
	1·98	100	76	100	96
	2·12	94	70	100	98
Control	—	0·7*	0·7*	0·7*	0·7*

* Figures from 150 insects.

TABLE 2. *The toxicity to* Calandra oryzae *of a direct spray of* P31, *Odourless Distillate, and Pool burning oil at* 20° C.

Oil	Deposit (mg./sq.cm.)	'Mortality' (%) 24 hr.	'Mortality' (%) 3 days	Knock-down (%) 24 hr.	Knock-down (%) 3 days
P31	1·51	6	6	24	24
	1·73	30	30	62	62
	1·87	40	38	62	62
	2·09	52	52	88	84
	2·30	58	58	90	92
	2·52	85	83	98	98
	2·81	90	88	100	100
O.D.	1·47	8	8	30	8
	1·70	16	16	44	22
	1·94	43	41	77	50
	2·09	44	46	72	52
	2·30	62	62	92	68
	2·56	40	28	84	46
	2·78	68	63	92	73
P.B.O.	1·50	12	14	18	16
	1·72	2	2	10	2
	1·86	6	4	12	4
	2·08	4	10	22	14
	2·29	38	38	60	44
	2·51	42	36	68	62
	2·79	28	36	56	42
Control	—	0·7*	2·0*	0·7*	2·0*

* Figures from 150 insects.

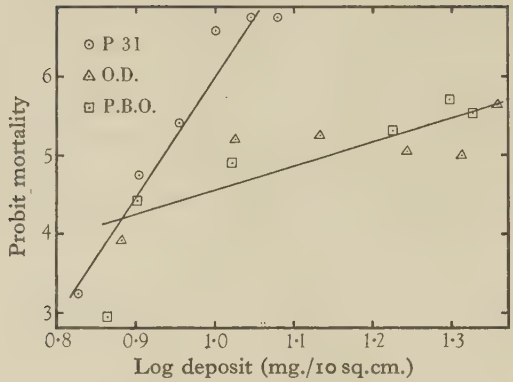

Fig. 2. The toxicities of direct sprays of P31, O.D. and P.B.O. to *C. granaria*.

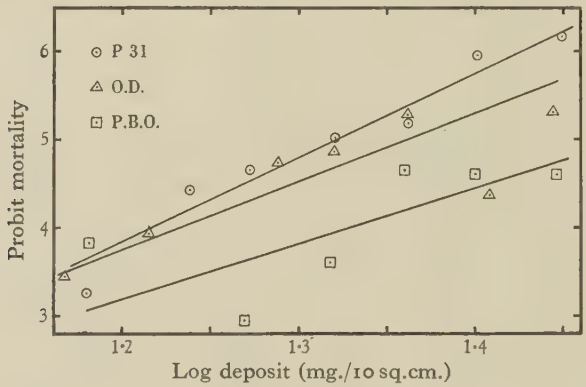

Fig. 3. The toxicities of direct sprays of P31, O.D. and P.B.O. to *C. oryzae*.

The following are general points shown by the data of Tables 1 and 2:

(1) The term 'mortality' has not its usual implication of irreversibility, for the percentage 'mortality' decreased considerably between 24 hr. and 3 days after spraying the batches of *C. granaria* with O.D. or P.B.O.

(2) The results for P31 are much more homogeneous than those for O.D. and P.B.O. (see also Figs. 2 and 3).

(3) The percentage of *C. granaria* knocked down by P31 but not dead was near zero, while the corresponding percentage of *C. oryzae* was considerably greater. There was a considerable percentage of both species knocked down by O.D. and P.B.O. but not dead.

(4) The changes in the corresponding percentages between 24 hr. and 3 days may be summarized as follows:

	C. granaria		*C. oryzae*	
	Knock-down	Mortality	Knock-down	Mortality
P31	Nearly constant	Constant	Constant	Constant
O.D.	Constant	Fell	Fell	Nearly constant
P.B.O.	Fell	Fell	Fell	Nearly constant

The relative toxicities of the three oils at higher dosages can be summarized as follows:

	Knock-down	Mortality
C. granaria	24 hr. P31 = O.D. > P.B.O. 3 days P31 = O.D. > P.B.O.	(O.D. ≯)* P31 > P.B.O. P31 > O.D. = P.B.O.
C. oryzae	24 hr. P31 = O.D. > P.B.O. 3 days P31 > O.D. = P.B.O.	P31 = O.D. > P.B.O. P31 > O.D. > P.B.O.

* Limited deduction owing to heterogeneity in the results.

In the above synopsis, '=' should be read as 'did not differ significantly in toxicity from', and '>' as 'was more toxic than'. It is evident that, irrespective of the species, the period between spraying and assessment of results, or whether toxicity was judged by knock-down or mortality, P31 was more toxic than P.B.O. On the other hand, O.D. ranged in toxicity from equality with P31 to equality with P.B.O. according to the different circumstances. This relation may be expressed as

$$\text{P.B.O.} < \text{P31} \geqslant \text{O.D.} \geqslant \text{P.B.O.}$$

Figs. 2 and 3 show graphically the mortalities obtained 3 days after spraying, for *C. granaria* and *C. oryzae* respectively. These figures demonstrate more clearly than the corresponding data in Tables 3 and 4 that *C. granaria* was more susceptible than *C. oryzae* to each of the three oils. It should be noticed that in Fig. 2 the slopes of the regression lines for the light oils were less than that for P31, and a tendency in this direction is also noticeable in Fig. 3.

Film tests

At 20° C., *Calandra* beetles, when confined on films of P31 on Whatman no. 544 filter-paper, climbed on top of one another and remained inactive, causing great variation in the doses of oil received by the individual insects of a batch. This effect was not observed when beetles were confined on treated filter-paper at 25° C., nor on brick or sacking at 20° C. The resistances of the two species to P31 were therefore compared by exposing batches of fifty beetles at 25° C. and 70% R.H. on no. 544 filter-papers sprayed with different deposits of the oil. The numbers of dead beetles were recorded after 3 days' exposure on the films, and the results are shown graphically in Fig. 4, in which each point represents the mortality observed in one batch of fifty insects. Mortalities of zero, which occurred at the lowest deposits, are not included in Fig. 4. The control mortalities for *C. granaria* and

C. oryzae were 0 and 5% respectively. Fig. 4 shows that *C. granaria* was more susceptible than *C. oryzae* to P31 films, a result which agrees with those for the direct spray tests (see Fig. 1).

To discover whether films of P31 were likely to be sufficiently toxic for the practical control of *Calandra* spp., *C. granaria* were exposed on films formed on Fletton bricks by P31 and the P31/water emulsion. Pyrethrum films on this substrate are slightly toxic (see Parkin & Hewlett, 1946). Brick samples were sprayed with deposits of up to 15 mg./sq.cm., i.e. far in excess of those attainable in practice, and the beetles were confined on the films 24 hr. later at 20° C. and 70% R.H. In

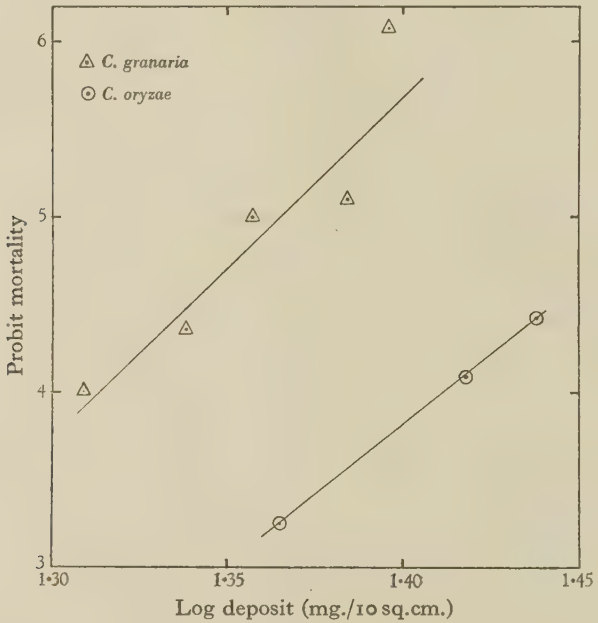

Fig. 4. The toxicity to *Calandra* spp. of a film of P31 on Whatman no. 544 filter-paper.

spite of the high deposits, however, after the beetles had been exposed on the films for 4 days the maximum mortality was only 6%.

Although the films on brick were of low toxicity, it was thought that films on sacking might well be highly toxic, as droplets of P31 remain suspended from projecting fibres of sprayed sacking, giving conditions of dosage of the insects different from those on porous, non-fibrous materials. Parkin & Hewlett (1946) have shown that pyrethrum films on sacking are of moderate toxicity. Samples of sacking were sprayed with about 2·5 mg./sq.cm. of P31, a level of deposit attainable in practice by spraying heavily. Batches of *C. granaria* were confined on the

samples 24 hr. after spraying. The experiment was carried out at 20° C. and 70% R.H. After the beetles had been exposed on the samples for 3 days, the mortality was only 1%.

Another experiment was undertaken to discover whether films of P31 on cement were toxic to *C. granaria*, and whether the toxicity of P31 films on cement could be increased by pretreatment with gelatin. Pretreatment of cement with gelatin has been shown by Hewlett & Parkin (1947) to increase the toxicity of pyrethrum films. Two cement samples pretreated with 5% w/v gelatin (edible grade) and two samples not pretreated were sprayed with approximately 2·5 mg./sq.cm. of P31. Fifty *C. granaria* were confined on each sample 24 hr. after spraying. The test was carried out at 25° C. and 70% R.H., the conditions of the experiments on pretreatment by Hewlett & Parkin (1947). After confinement on the films for 3 days the mortality on the pretreated samples was 92 and 100%, while on those not pretreated it was 0 and 2% respectively. This showed that the films on the pretreated cement were of high toxicity, but those on non-pretreated cement were non-toxic.

Recovery from P31 *poisoning*

In the direct spray tests, the percentage knock-down due to P31 remained constant or nearly constant between 1 and 3 days after spraying. In these tests, however, many of the beetles would have received doses of P31 considerably above the minimum necessary to knock them down. An experiment was therefore carried out to discover whether beetles were likely to recover when given doses of P31 little in excess of those sufficient to knock them down.

Batches of fifty beetles were enclosed on films of P31 on Whatman no. 1 filter-papers 7 cm. in diam. The *C. granaria* were exposed on filter-papers each treated with 0·25 ml. of oil, but the *C. oryzae*, because they were more resistant, were exposed to 0·30 ml. per filter-paper. The insects were examined half-hourly, and those knocked down removed to clean filter-papers. After knock-down on the films had proceeded for 6 hr., by which time the cumulative knock-down of the two species was 66 and 82% respectively, the beetles remaining on the films, i.e. not knocked down, were discarded. The rest of the beetles were observed at intervals to discover how many recovered, and were classified as dead, affected (knocked down but not dead) and normal. The whole experiment was carried out at 25° C. and 70% R.H.

Among the 201 *C. granaria* removed from the oil films, maximum recovery occurred on the fourth day after treatment, when 12% were normal, 5% affected and 83% dead. Among the 246 *C. oryzae* removed from the films, maximum recovery occurred on the first day after treatment, when 4% were normal, 17% were affected and 79% were dead. It therefore appears that only a small proportion of either species is likely to recover completely from doses of oil sufficient to knock the beetles down.

Discussion

Shepard (1942) considered that the toxic action of petroleum oils on insects was due to (1) blockage of the spiracles, and consequent suffocation, or (2) penetration of the tissues and breakdown of the tissue structure by the oils, or (3) a fumigant action by the volatile components of the oils. The action of P31 on *Calandra* has not been thoroughly investigated, but probably P31 kills the beetles by blocking their spiracles and/or tracheae, so suffocating them. (2) seems unlikely, because the large molecules of P31 probably would not readily pass through the cuticle, and P31 is chemically inert. (3) seems unlikely because P31 is of very low volatility. Oil was found in the tracheae of many individuals of batches of beetles knocked down by P31, but it may have been present in the tracheae of the individuals in which it was undetected, because, even when dyed, it is difficult to see in the tracheae. Shepard deduced that, if oils kill an insect by suffocation, there will be an optimum viscosity for toxicity. There is probably an optimum viscosity for petroleum oils for the control of *Calandra*. P31 is more viscous than O.D. and P.B.O., but less viscous than liquid paraffin; and it was always more toxic than P.B.O., often more toxic than O.D., and more toxic to *C. granaria* than liquid paraffin. Shafer (1911) found that the hearts of insects beat for a long time after the insects had been immobilized by suffocation; the hearts of *Calandra* beat for 3–5 days after the beetles had been completely immobilized by P31. Finally, beetles put into an atmosphere of nitrogen developed after 2 or 3 min. the same symptoms as in P31 poisoning.

The modes of action of O.D. and P.B.O. were not investigated.

Direct sprays of P31 show considerable promise for the control of *C. granaria*. A direct spray can be effective, of course, only in situations where the great majority of the beetles are exposed, and not hidden in cracks, etc. These conditions often occur, in, for example, silo-bins, barges, farm granaries, and empty stowages in warehouses. Very heavy direct sprays of P31 might be effective against *C. oryzae*. P31 shows more promise than O.D. or P.B.O., because it was often more, and never less, toxic, and is non-inflammable. Films of P31 on absorbent surfaces are likely to be useless against either species, unless, before being sprayed, the surfaces are treated with some substance such as gelatin.

This work has formed part of the research programme of the Pest Infestation Laboratory, and is published by permission of the Department of Scientific and Industrial Research. Miss B. Clayton gave considerable assistance in the experimental work.

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RELATION BETWEEN PARTICLE SIZE AND SHAPE OF INSECTICIDAL SUSPENSIONS AND THEIR CONTACT TOXICITY

I. D.D.T. SUSPENSIONS AGAINST *TRIBOLIUM CASTANEUM* Hb.

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(With Plate 16 and 7 Text-figures)

Fairly coarse aqueous D.D.T. suspensions can be made by the exchange of solvent method. By varying the method of preparation the mean size of crystal in suspension can be altered. The principles involved are outlined, and methods and apparatus are described for the preparation of the following suspension types: colloidal D.D.T., a suspension containing elongated hexagonal plates, a suspension of somewhat elongated two-dimensional aggregates of plate-like crystals, and three suspensions of needle-shaped crystals of different lengths. All the D.D.T. crystals are of the same fundamental crystal habit. These suspension types were selected to study the effect of both crystal size and shape on toxicity.

The suspensions were tested against *Tribolium castaneum* Hb., using a recently developed dipping technique. It was shown that the results obtained were independent of any operation peculiar to the technique.

Suspensions tested by a spraying method gave data similar to those obtained by dipping, except for certain anomalous results obtained where the crystals were considerably broken up in passing through the spray nozzle.

Toxicity differences were assessed by the probit method. The lines obtained for the crystalline suspensions were generally parallel to one another, but the colloidal D.D.T. gave a line differing in slope from the others.

The dipping tests showed that, within the range of crystal sizes up to about 400 μ, toxicity increased with increasing needle length; breadth was less important, but a suspension of needle-shaped crystals was as toxic as one containing considerably larger plate-shaped crystals at the same weight/volume concentration. Colloidal D.D.T. was less toxic than any crystalline suspension. Thus, taking the median lethal concentration for 400 μ needles as 100, the values for the other types were: for 120 μ needles, 210; for plate aggregates (240 × 140 μ), 260; for 40 μ needles, 390; for 60 × 15 μ plates, 510; and for colloidal D.D.T., 1700.

These differences in toxicity were largely paralleled by retention of greater amounts of D.D.T. from the coarse suspensions than from finer ones; two extreme types were of almost identical toxicity, comparing the percentage kills produced by equal amounts of poison retained, and not by equal concentrations in suspension. The methods of carrying out these retention tests are described.

Introduction

It is difficult to make any generalizations on the effects of particle size on toxicity. The effects must depend on the physical system under consideration (aerosol, suspension, etc.), and will vary for different poisons and different test subjects.

Little has been published on the effects of particle size on suspensions of solid contact poisons. Martin (1940) considers that a suspension of rotenone crystals would be less toxic than a system containing rotenone in 'a high state of suspension in the presence of resins inhibiting crystallization'.

The information on D.D.T. is even more scanty. Starr (1945) has described a method of spraying a solution of D.D.T. in alcohol, acetone or dioxane from one nozzle and water from another. The two sprays mix in the air and the D.D.T. is solid by the time it reaches the sprayed surface. It is possible to vary the 'type of particle' by varying the solvent and the concentration of D.D.T. No mention is made of the type of particle produced, nor of the effect of particle type on the retention or toxicity of the D.D.T.

In the work described below a detailed study has been made of the effects of crystal size and shape on contact toxicity of simple aqueous D.D.T. suspensions. These have been prepared by a method (exchange of solvent) which utilizes the principle employed by Starr but controls the conditions of preparation more rigidly, giving various characteristic types of suspension.

Principles involved in the preparation of suspensions

General methods

D.D.T. being insoluble in water is used in spraying either as an emulsion in oil or as a suspension. Commercial suspensions contain relatively large amounts of clay, detergent, etc. For experimental purposes less complex suspensions can be prepared by diluting a small volume of a water-miscible solvent containing D.D.T. with a larger volume of a dilute aqueous detergent solution. The physical conditions of dilution, and, in some cases, those of storage in the period immediately following, determine the shape and size of the microcrystals obtained. By controlling these conditions it is possible to prepare suspensions of widely different crystal size and shape but of the same crystal habit.

By such methods, suspensions of six different types have been prepared. To simplify measurements of crystal size, attempts were made to choose methods of preparation such that the crystals in each suspension were, as nearly as possible, of uniform dimensions. As will be seen, these attempts were only partially successful.

The variable factors involved in suspension preparation are: the D.D.T. solvent employed, the concentration of D.D.T. in it, and the percentage of solvent in the mixture; the detergent and its concentration in the mixture; the rate of admixture, the temperature and rate of stirring during admixture, and the temperature of ripening (see below) after admixture. To simplify toxicity comparisons, crystals of different dimensions should be obtained in media as similar as possible. For this reason, the adjuvants used have been restricted to two solvents, acetone and ethyl alcohol, and to one detergent, Sulphonated Lorol, a commercial product, of which

the main constituent is sodium dodecyl sulphate. Addition of the solvent solution to the aqueous detergent solution, or the reverse, gives two general and equally useful methods of preparation. The different variables mentioned above do not have the same relative importance in each method. As a different procedure is needed for each, they will be considered separately.

Addition of D.D.T. solution to aqueous detergent solution

Crystal growth

This method is more closely analogous than the second to the preparation of inorganic salt precipitates by double decomposition from two aqueous solutions. The formation of precipitates in this way can be divided into three stages described by Mees (1944) in summarizing the work of Sheppard & Lambert (1926, 1928) and others on silver bromide precipitation in the presence of gelatin.

The first stage, immediately after mixing, involves a highly supersaturated solution in which a very large number of ultramicroscopic crystal nuclei form and then grow until supersaturation no longer exists.

The second and third stages are associated with flocculation of these nuclei. In the second, the nuclei join to form larger units, while the more soluble small crystals dissolve and redeposit material on the larger (Ostwald ripening). With silver bromide this produces crystals of microscopic size.

In the final stage, these crystals fuse together to form larger crystals.

The factors (concentration of reacting solutions, solubility of precipitate and time) controlling crystal size under these conditions have been described by von Weimarn (1925–6), who gives three laws of precipitation, and a corollary derived from the second and third. As will be seen below, the first two, which deal with the effect of different concentrations of reacting solutions, do not apply in this investigation. The third law states, in effect, that the less soluble a precipitate is in the medium in which it is formed, the smaller will be the crystals produced. Considering unit volume, the number of nuclei or degree of dispersion (δ) obtained in the first stage is expressed by

$$\delta = \frac{J(Q-L)}{L},$$

where Q = total amount of precipitating substance available in solution, L = solubility of large crystals of precipitate, and J = constant for a system under given conditions. Thus $Q-L$ = extent of supersaturation produced on mixing.

This solubility factor also applies to the two growth stages. The rate at which these proceed increases with the solubility of precipitate in the medium. For massive crystals this is never, of course, large, but for small particles it may be considerable. If the solubility can be made low enough, the growth stages can be cut down or eliminated (Sheppard & Lambert, 1928). Heat treatment to increase the solubility is widely used in analytical chemistry for 'ageing' precipitates. Other means of increasing the solubility will also favour increased growth.

Application to preparation of D.D.T. suspensions

A situation somewhat similar to that described by Mees (1944) exists when a solution of D.D.T. in acetone or alcohol is added to an aqueous detergent solution. However, the D.D.T. precipitate is not produced by double decomposition, and the degree of supersaturation attained by addition of each drop of solvent solution is more nearly constant over the period of addition. The D.D.T. crystals produced in this way, although much larger, behave in a way similar to crystals of silver bromide.

In this method it is not practicable, nor very useful, to alter some of the variables listed above. The concentration of Sulphonated Lorol cannot be increased beyond a certain point (about 0·5%), at which it begins to show a slight toxicity to insects. It is necessary, except in one case shortly to be described, to have some detergent present during admixture in order to obtain regularity of crystal growth. Moreover, detergent is required in the test mixture to ensure even wetting and dosage of the insects. The concentration of Sulphonated Lorol is therefore kept constant at 0·1%. In a simple suspension it is not always feasible to include more than 0·2% D.D.T. in the final mixture, as it is liable to coagulate during precipitation. (In some cases the D.D.T. can, if necessary, be concentrated by sedimentation.) Further, to obtain sufficient toxicity, the D.D.T. concentration must not be much below 0·1%. For these reasons it was decided to prepare suspensions in which the Sulphonated Lorol and D.D.T. concentrations are both fixed at 0·1%. Von Weimarn's (1925–6) first and second laws are therefore not relevant. To avoid toxicities in the dipped controls, the solvent concentration must not be more than 10% in the final mixture.

The figures given refer to the composition of the suspensions as used for biological tests. It was in some cases necessary to depart from these conditions for a time during preparation of the suspensions, but the concentrations were later adjusted to the above composition.

During mixing the system must be stirred continuously, but the rate of stirring, above a certain minimum, is not critical. Small variations in the concentrations of D.D.T. and Sulphonated Lorol around 0·1% are also unimportant in determining the crystal dimensions. The other factors—percentage of solvent in the mixture, rate and temperature of mixing and temperature of ripening—must be carefully controlled.

First type of suspension

It has been mentioned above that growth can be checked at the first stage if the solubility of the disperse phase can be made low enough. This can be achieved by using the minimum quantity of ethyl alcohol (a poor D.D.T. solvent) for making the D.D.T. solution, and mixing rapidly at a relatively low temperature, as these conditions correspond to a high value of $(Q-L)$ in von Weimarn's equation. Provided the temperature of ripening is no greater than room temperature, a rather unstable colloidal suspension results. Detergents, even in very low concentrations (0·1% or less), exert a considerable solubilizing effect on organic compounds

normally insoluble in water (McBain, 1942). The Sulphonated Lorol in the above mixture encourages growth, which takes place slowly. If the alcohol solution is mixed with water in the absence of detergent, a more stable colloidal suspension results, and can be kept without growth for several hours. Growth is so slow that toxicological tests can be made before it becomes significant. However, since some detergent must necessarily be present during the biological tests, it was added immediately prior to each test. No particle-size measurements were carried out on the colloidal D.D.T. suspension.

Second type of suspension

By making the final concentration of solvent (acetone) 10%, adding the acetone slowly to the 0·1% detergent solution at 40° C., and allowing the mixture to ripen overnight at 27° C., the very uniform second type of suspension is obtained. The crystals are elongated hexagonal plates, about $60 \times 15\mu$.

It is not possible to increase the temperature of admixture much above 45° C., owing to loss of acetone by evaporation. The temperature of ripening (using a sealed vessel) can be increased to 60° C. or more. This results in less uniform but larger plate-like crystals. An increase in the speed of addition also reduces the uniformity of the crystals.

In this preparation the D.D.T. passes through the first two stages of growth, i.e. formation and growth of nuclei from supersaturated solution, followed by ripening. Growth is encouraged by the presence of 10% solvent, the relatively high temperatures of mixing and ripening, and by the solubilizing properties of the detergent. The solubility of D.D.T. is not great enough to allow the third stage to be reached.

Third type of suspension

If, however, the solubility of D.D.T. is raised by increasing the amount of acetone in the mixture to 20%, while otherwise carrying out the preparation as for $60 \times 15\mu$ plates, the third stage of growth takes place to some extent. It is not, however, complete, but can be carried further by increasing the temperature of ripening to 60° C., the mixture being, of course, kept in a sealed container. This results in plate-like crystals, individually a little longer than those obtained before (about 70×15–20μ), but fused together in two-dimensional aggregates. These are much less regular in outline and size than the $60 \times 15\mu$ plates, but such a suspension proved useful for toxicological tests, and, when the acetone concentration has been adjusted to 10%, to correspond with other suspensions, gives the third of the six types tested. The size of the aggregates is roughly $240 \times 140\mu$.

Addition of detergent solution to D.D.T. solution

Controlling factors

In the above method, the D.D.T. is precipitated in consecutive small amounts as each drop of the solvent solution reaches the aqueous solution, and precipitation

continues throughout the period of admixture. If the order of addition is reversed, and the aqueous solution of Sulphonated Lorol is gradually added to the organic solvent solution, no precipitation takes place at first. When the D.D.T.-solvent power of the alcohol- (or acetone-) water mixture has been reduced to a certain value, precipitation takes place rapidly and is completed after the further addition of a relatively small amount of water. There is little or no supersaturation. The crystals grow very rapidly to a length of not less than 30μ, with no after-growth. Consequently the temperature of precipitation or ripening and the percentage of solvent are unimportant in determining the crystal size.

As before, it is convenient to keep the concentration of Sulphonated Lorol and D.D.T. in different suspensions constant at 0·1 %, and to limit the percentage of solvent to 10%. Further, small variations on either side of these values again do not have any pronounced effect on the crystal form. The two factors to be carefully controlled are the rate of addition and the rate of stirring during precipitation.

As mentioned, the D.D.T. is precipitated quickly, giving needle-shaped crystals.

Fourth, fifth and sixth suspension types

If the rates of addition and stirring are low, long needles are formed; they are apparently aggregates of smaller units. If, however, the rates of addition and stirring are increased, the aggregates do not form and shorter needles result. By these methods, and using acetone as solvent, two types of needle suspension are obtained—one with aggregates about 400μ long, and the other with needles of about 120μ. By substituting alcohol for acetone and with high rates of addition and stirring, a third type, containing needles about 50μ long, is produced.

Techniques for preparing suspensions

Materials

Pure recrystallized *p*, *p'*-D.D.T., m.p. 108·5–109·5° C., and distilled water were employed throughout. The concentrations of D.D.T. and Sulphonated Lorol are given as w/v %, and the concentrations of acetone and alcohol as v/v %.

First three types of suspension

Apparatus

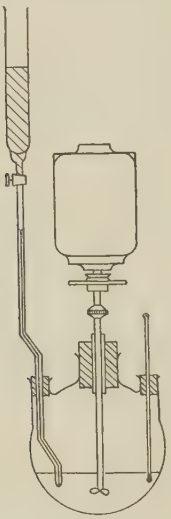
A 1 l. three-necked flask was fitted with a thermometer, a dropping funnel and an electrically driven glass stirrer running in a greased glass bearing held in the central cork. The dropping funnel was fitted with a tap and a length of capillary tubing ending in a carborundum-ground point placed 1 cm. below the level of the liquid. The flask could be immersed in a water-bath at 40° C. (see Text-fig. 1).

Type I (colloidal D.D.T.)

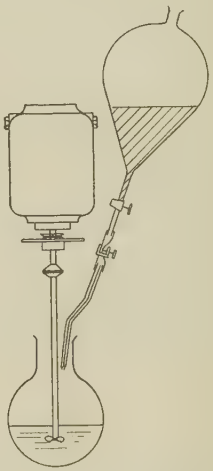
As will be seen below, it was necessary to have this type at a D.D.T. concentration of 0·2 % instead of 0·1 %, as with the other types. Further, as the Sulphonated Lorol

was added just prior to the biological tests, it was necessary to prepare the colloid at a strength slightly greater than 0·2% and later add a small volume of concentrated Sulphonated Lorol solution to bring the D.D.T. concentration to 0·2% and the detergent strength to 0·1%.

In the flask was placed 165 ml. of water at room temperature. By boiling in a test-tube, 0·4 g. of D.D.T. was dissolved in 10 ml. alcohol and transferred to the dropping funnel while still warm. With the stirrer running at moderate speed and the tap fully open, the alcohol solution was run into the water as rapidly as possible. A little coagulation of D.D.T. normally took place, to give a small deposit on the flask walls at the surface of the liquid. The main bulk of the colloid was transferred



Text-fig. 1. Apparatus for preparing suspensions of types I, II and III.



Text-fig. 2. Apparatus for preparing suspensions of types IV, V and VI.

to another flask, with thorough draining. Using two successive 2·5 ml. portions of warm alcohol, the test-tube and funnel were then washed, and the washings transferred to the three-necked flask. By gentle heating over a small flame, the deposit of D.D.T. on the flask was dissolved, and, while the alcohol was still hot, the colloidal suspension was poured back into the three-necked flask. This gave a suspension free from any deposit. The total volume was 180 ml. containing 0·4 g. D.D.T. (0·22%) and 15 ml. of alcohol (8·3%).

Type II (60 × 15 μ *plates*)

The flask, containing 0·2 g. Sulphonated Lorol dissolved in 180 ml. water, was immersed in the water-bath at 40° C. and the stirring motor started. The dropping funnel was charged with 0·2 g. D.D.T. dissolved in 20 ml. acetone. When the

detergent solution had reached 40° C., acetone solution was added at 60 drops per min. by raising the funnel, so that the tip was clear of the liquid, adjusting the tap, and reimmersing the tip of the funnel. From time to time the speed was checked in the same way. Addition was complete in about 30 min.

The milky suspension was transferred to a stoppered flask and kept at 27° C. overnight, to permit crystal growth. Sedimentation was normally complete by the morning.

The suspension contained D.D.T. 0·1%, Sulphonated Lorol 0·1% and acetone 10%.

Type III (*plate aggregates*)

0·2 g. of Sulphonated Lorol, dissolved in 160 ml. of water, was placed in the flask, in the water-bath at 40° C. The funnel contained 0·2 g. D.D.T. in 40 ml. acetone. When the solution in the flask had reached 40° C., the acetone solution was added at 240 drops per min., and addition was complete in 15 min. The suspension was quickly transferred to an 8 oz. bottle with a tightly fitting greased glass stopper. The stopper was sealed by several strips of insulating tape, and the bottle kept at 60° C. overnight, after which it was removed and cooled to room temperature with occasional vigorous shaking. When the aggregates formed in the mixture at 60° C., they were rather large and unstable, and had bubbles of vapour adhering to them. The shaking broke them down to a stable size and removed the bubbles and the aggregates settled down to form a bulky sediment.

When sedimentation was complete, rather more than half the supernatant liquid was cautiously withdrawn, using a filter-pump and trap, and the acetone content was adjusted to 10%. The final composition was thus D.D.T. 0·1%, Sulphonated Lorol 0·1% and acetone 10%.

Second three types of suspension

Apparatus

All three preparations were carried out at room temperature. The solvent solution was contained in a 250 ml. round-bottomed bolt-head flask fitted with an electrically driven glass stirrer. A dropping funnel was arranged by connecting a 500 ml. separating funnel to a short length of bent glass capillary tubing, ending in a jet. The connecting rubber tubing carried a screw clip, as shown in Text-fig. 2.

Type IV (*needle aggregates*)

A solution of 0·2 g. Sulphonated Lorol in 180 ml. water was placed in the separating funnel. The bolt-head flask was removed and the passage of solution through the jet was adjusted by the screw clip to 30 drops per min. 0·2 g. D.D.T., dissolved in 20 ml. acetone, was transferred to the bolt-head flask, now in position again. Slow stirring (about 300 r.p.m.) was now started and the tap opened. A constant check was kept on the speed of admixture. Precipitation began at about

the 15th minute and was certainly complete at the 25th, at which time the screw clip could be opened fully, allowing the Sulphonated Lorol solution to enter in a continuous stream. In this way mixing was complete in about 50 min. The mixture contained 0·1% D.D.T., 0·1% Sulphonated Lorol and 10% acetone, and was kept stoppered till required.

Type V (*short needles from acetone*)

Again 0·2 g. Sulphonated Lorol was dissolved in 180 ml. water, and the speed of mixing was adjusted to 200 drops per min. 0·2 g. D.D.T. in 20 ml. acetone was contained in the flask as before. Stirring was carried out at the maximum speed obtainable (about 2000 r.p.m.). The depth of the paddle was adjusted so that loss by spirting was negligible. Precipitation started about 2 min. after the tap was opened. At about the 10th minute, when precipitation was complete, the rate of stirring was moderated to avoid excess foaming. The tap was opened fully and addition continued till complete (about 30 min. from the start). Again the mixture contained 0·1% D.D.T., 0·1% Sulphonated Lorol and 10% acetone.

Type VI (*short needles from alcohol*)

This preparation was very similar to the previous. Absolute alcohol was substituted for acetone, and the Sulphonated Lorol solution was added in a continuous stream from the capillary jet. Precipitation began in under 1 min. Vigorous stirring was continued for 5 min. and then moderated as before. Addition was complete in 20 min.; the composition was D.D.T. 0·1%, Sulphonated Lorol 0·1% and alcohol 10%.

Variability in preparation of suspensions

A certain amount of day-to-day variation in microcrystal size was noted with each of these methods of preparation, and small changes occurred in some variables normally regarded as fixed. Rate of stirring and rate of mixing could not be as accurately controlled as some other variables. Moreover, the exact shape of the stirring paddle used in the last two preparations, which involved vigorous stirring, seemed to make some difference to the size of the needles produced. The suspensions used in the preparation of the photomicrographs were prepared at a much later date than those used for biological tests, and a different paddle was used. For this reason the needles shown in Pl. 16, figs. 4 and 5, are slightly longer than those actually used for the biological tests. Also, the aggregates shown in Pl. 16, fig. 2, are slightly larger than those used in the tests.

Method of size estimation

The mean size of crystal in the suspension (except the colloid) was roughly estimated by measuring about twenty random crystal samples in a drop.

With $60 \times 15\mu$ plates, the crystal size was sufficiently constant for repeated measurements to be omitted.

Most of the tests using suspension type IV (needle aggregates) were done before the importance of needle length in determining toxicity was fully realized, and the crystal size was defined by method of preparation rather than by measurement. More accurate measurement of the length of needles in types V and VI was required, as it was necessary to compare their toxicity with that of plate-shaped crystals of approximately the same size. No plate-shaped crystals were obtained of length comparable with that of the needle aggregates, and as the variation in length from one preparation to another was not great, measurements of needle aggregate length were not generally made, even in the later experiments. The mean length was about 400μ.

With plate aggregates the maximum length and breadth were taken.

Toxicity tests

Dilutions

To obtain a probit line for each suspension type, a series of about six concentrations was required. These were arranged logarithmically, the ratio of each to the next higher concentration normally being 0·7. The dilution medium in each case corresponded to the final composition of the particular suspension type.

Methods

The method chiefly used was a recently developed dipping method, but some tests have been carried out using modifications of the dipping technique, and also the Potter spraying tower. These tests were to ensure that the results obtained by the standard dipping technique were general and not dependent on any operation or series of operations peculiar to the dipping method.

Suspensions of types I, II and VI can be tested using the spraying apparatus (Potter, 1941), but crystals in the other three types are broken up into smaller units in passing through the nozzle of the tower. Use of a dipping method (McIntosh, 1947) ensures that the insects are completely immersed in the toxic medium, so that the crystals reach them intact.

Dipping technique

The test subjects were *Tribolium castaneum* Hb., reared on wholemeal flour. The temperatures of rearing, of dipping and of storing after treatment were all kept at 27° C. to ensure reproducible results (Potter & Gillham, 1946). The insects were reared and stored in a constant-temperature room, near which the dipping apparatus was placed.

The dipping method is a slow one, and it was not normally possible to obtain more than two probit lines in a day. Except for colloidal D.D.T., the suspensions were prepared the evening before dipping and stored in the constant-temperature room overnight. Dilutions were usually made the next morning. The insects were removed from their culture during the early morning and transferred to clean

empty specimen tubes, fifteen to a tube. The tubes were removed one at a time from the constant-temperature room immediately before treatment, and the (labelled) dipping tubes were put back again immediately after.

The dipping period was 3 min. At each concentration, 8 ml. of suspension was used for three successive batches of fifteen insects. The suspension was then rejected and 8 ml. of the next concentration used in the same way. Three replicates were obtained as a control, using the dilution medium alone.

Any insects found adhering to the stoppers on their removal from a dipping tube were rejected.

The first series of concentrations was tested in the morning, the second in the afternoon. The insects were kept in separate batches for 2 days at 27° C. before inspection.

Successive groups of three tubes at a time, chosen at random, were removed from the first batch to the laboratory and the contents of each tube inspected, using the warm-plate technique (Tattersfield & Potter, 1943). Each insect was classified as normal (N), slightly affected (S), badly affected (B), moribund (M) or dead (D). The figures for the replicates of each concentration were combined and the kill taken as $B+M+D$. To ensure that decisions were not biased, the number on the tube being inspected was not noted until all fifteen insects had been classified.

Results of toxicity tests

Treatment of results

From the dosage-mortality relationships obtained by the above methods, straight lines were obtained by standard methods of probit analysis, showing the relationship of probit kill to log of percentage concentration. From such lines the median lethal doses were calculated.

It should be emphasized that no absolute values for median lethal dose can be given. The actual kill produced by any particular preparation depends both on the method of testing and on the various stages of the technique. The kill would be expected to vary with variation in temperature and duration of dipping or of storage between treatment and inspection. Consequently the absolute values of toxicity given below cannot be compared directly with values obtained using other testing methods.

Although the method had been carefully standardized, small day-to-day variations, both in median lethal concentration and in gradient of the probit line, frequently occurred. Some of these variations may be attributable to differences in the insect cultures employed; but, in addition, there were unexplained variations. The differences obtained between different suspension types were, however, quite consistent.

Results using the standard dipping technique

The different types of suspension were not tested in the same order as that in which the preparations are given, but, broadly speaking, in order of decreasing

toxicity. It has not been possible to test each type against every other, but enough tests have been done to establish the relative toxicities of the six types.

In the first place, needle aggregates were tested against $60 \times 15\mu$ plates, the needle aggregates being considerably more toxic.

Further tests were made to find whether the greater toxicity of the needle aggregates was due to their higher length:breadth ratio, or merely to their greater overall length. Suspensions of two types were prepared: first, one containing needles shorter than the needle aggregates, and secondly, one containing plate-shaped crystals of a larger size than $60 \times 15\mu$. For this purpose, short acetone needles were prepared. Their mean length, in the suspensions used in this series, was 114μ. It was not found possible, using only Sulphonated Lorol and acetone, to prepare a suspension containing plate-shaped crystals of a uniform size much greater than $60 \times 15\mu$. However, by causing plates of roughly this size to fuse together, the aggregates were formed. Although they did not have the same continuous structure as large individual plates, and were of a less regular outline, they served the same purpose. As mentioned above, the size of an aggregate was taken as its maximum length by maximum breadth; the mean size of aggregates in the suspensions used in this series was $244 \times 137\mu$. These two types (short acetone needles and plate aggregates) were found to have an almost identical toxicity, intermediate in value between the needle aggregates and the $60 \times 15\mu$ plates. The third size of needle (short alcohol needles) had a mean length of 39μ in the two suspensions used. This type was only very slightly more toxic than the $60 \times 15\mu$ plates.

Finally, the colloidal D.D.T. was much less toxic than any of the other types. For this reason it was desirable to have 0·2% as the top concentration instead of 0·1% as with the other types.

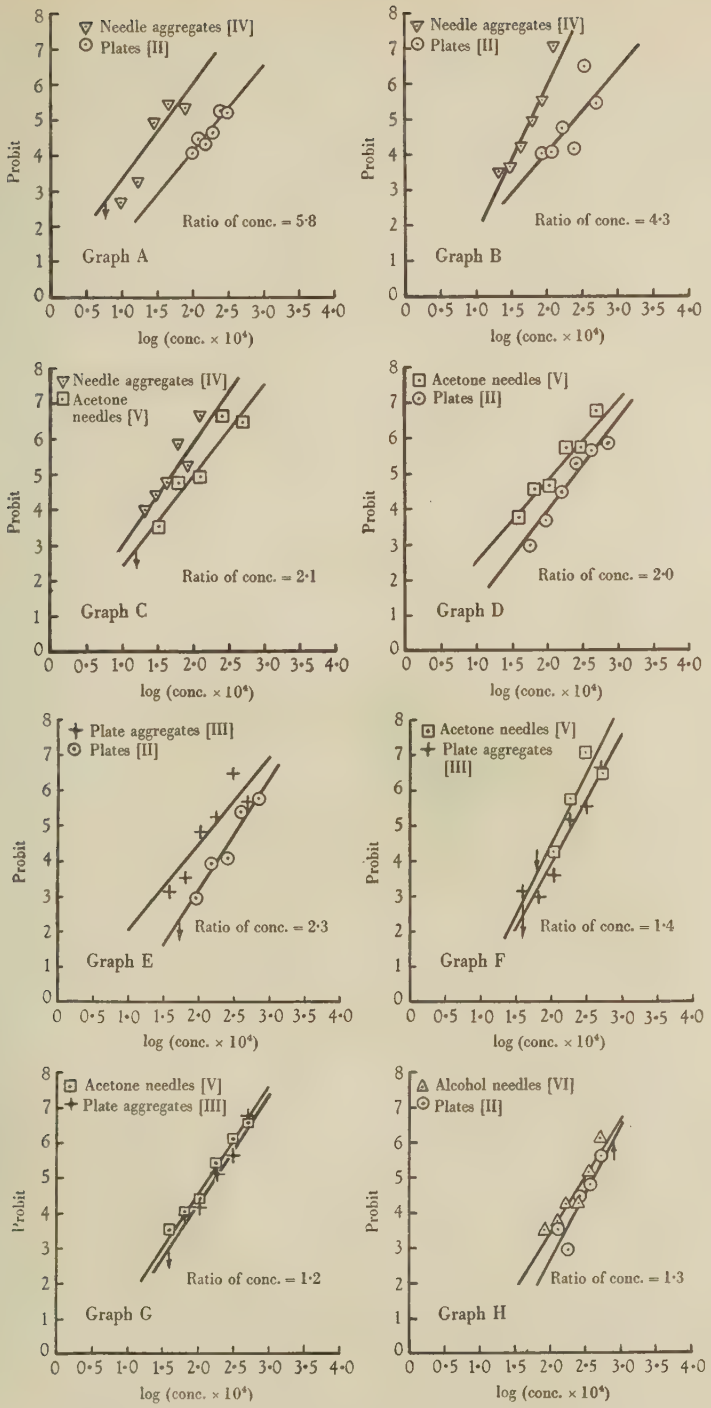
The last two suspensions mentioned (short alcohol needles and colloid) contained 10 and 7·5% alcohol respectively. To show that the toxicities of such suspensions are directly comparable to each other and to those of suspensions containing acetone, two suspension types ($60 \times 15\mu$ plates and needle aggregates, both of which sediment rapidly) were used in the following manner. The concentrated (0·1%) suspension was allowed to stand overnight so that sedimentation was complete. As much as possible of the supernatant liquid was drawn off, using a filter-pump and trap, so that its volume could be measured. Half of it was rejected and a like volume of solution was prepared, containing 10% alcohol instead of acetone. In the meantime the sedimented D.D.T. was shaken with the small volume of liquid remaining with it, and the mixture divided into halves. To one was added the 10% acetone solution, and to the other the 10% alcohol solution. The latter gave a suspension containing 10% solvent of which the bulk was alcohol. Each of these suspensions was now diluted with the appropriate medium and tested.

The results of all these tests are given in Text-figs. 3 and 4 and in Table 1. Each of the lines is the calculated 'best fit' for the group of points in question. The symbols $\uparrow$ and $\downarrow$ signify concentrations at which were obtained kills of 100 and

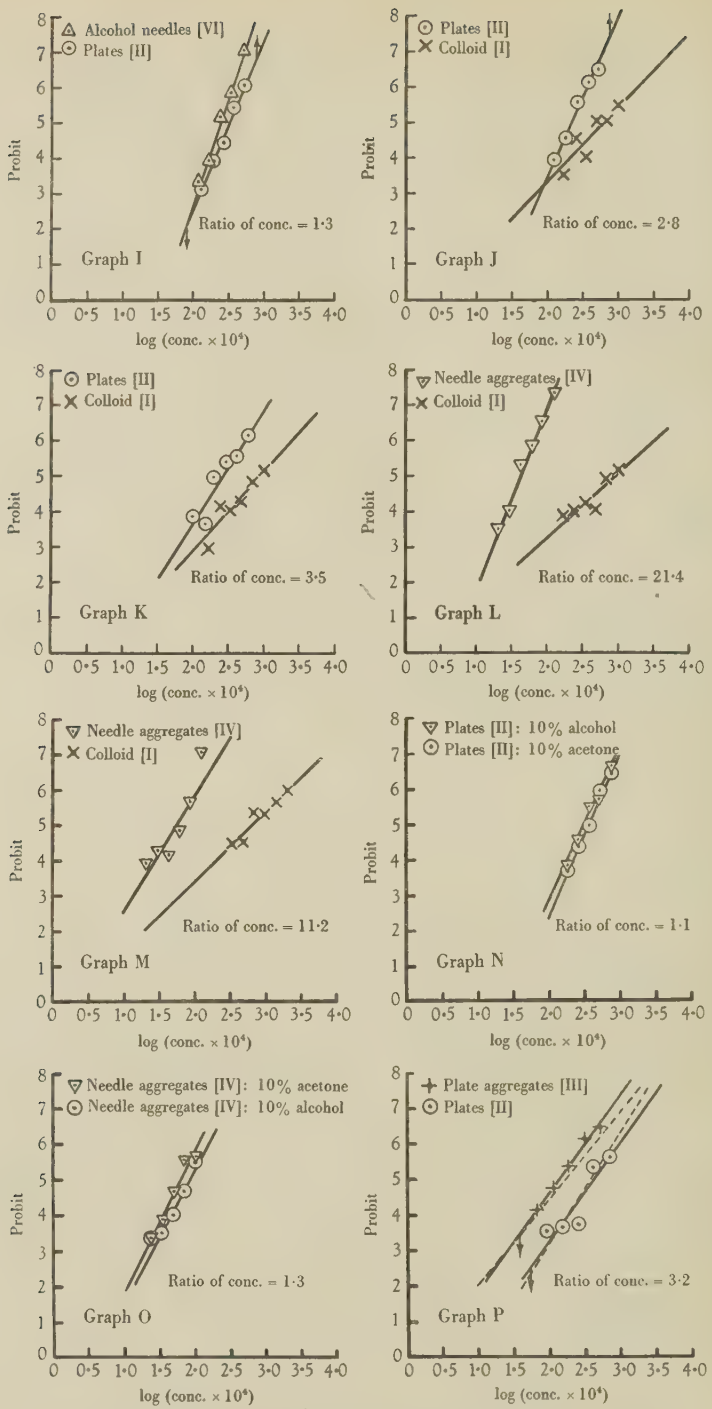
TABLE 1. *Results of tests A–O*

Test	Suspension type: Colloid I	60 × 15 μ plates II	Plate aggregates III	Needle aggregates IV	Short acetone needles V	Short alcohol needles VI	Types tested	Mean size in μ	No. of samples for measurement	Equation	Standard error in b	Whether parallel	Log [M.L.C. × 10^4]	Diff. in logs	Mean diff. in logs	Mean ratio of M.L.C.	Test
A	—	X	—	—	—	—	II	60 × 15	-	$y = 2{\cdot}42x - 0{\cdot}73$	$2{\cdot}42 \pm 0{\cdot}43$	Par.	$2{\cdot}37 \pm 0{\cdot}037$	$0{\cdot}76 \pm 0{\cdot}050$			A
	—	—	—	X	—	—	IV	—	—	$y = 2{\cdot}70x + 0{\cdot}64$	$2{\cdot}70 \pm 0{\cdot}11$		$1{\cdot}61 \pm 0{\cdot}035$		0·70	5·0	
B	—	X	—	—	—	—	II	60 × 15	—	$y = 2{\cdot}41x - 0{\cdot}80$	$2{\cdot}41 \pm 0{\cdot}35$	Not par.	$2{\cdot}41 \pm 0{\cdot}037$	$0{\cdot}63 \pm 0{\cdot}043$			B
	—	—	—	X	—	—	IV	—	—	$y = 4{\cdot}20x - 2{\cdot}46$	$4{\cdot}20 \pm 0{\cdot}42$		$1{\cdot}78 \pm 0{\cdot}023$				
C	—	—	—	X	—	—	IV	--	—	$y = 2{\cdot}87x + 0{\cdot}19$	$2{\cdot}87 \pm 0{\cdot}36$	Par.	$1{\cdot}68 \pm 0{\cdot}031$	$0{\cdot}32 \pm 0{\cdot}051$	—	2·1	C
	—	—	—	—	X	—	V	—	—	$y = 2{\cdot}54x - 0{\cdot}08$	$2{\cdot}54 \pm 0{\cdot}30$		$2{\cdot}00 \pm 0{\cdot}041$				
D	—	X	—	—	—	—	II	60 × 15	—	$y = 2{\cdot}57x - 1{\cdot}18$	$2{\cdot}57 \pm 0{\cdot}29$	Par.	$2{\cdot}40 \pm 0{\cdot}036$	$0{\cdot}31 \pm 0{\cdot}053$		2·0	D
	—	—	—	—	X	—	V	—	—	$y = 2{\cdot}27x + 0{\cdot}26$	$2{\cdot}27 \pm 0{\cdot}25$		$2{\cdot}09 \pm 0{\cdot}038$				
E	—	X	—	—	—	—	II	60 × 15	—	$y = 3{\cdot}14x - 3{\cdot}11$	$3{\cdot}14 \pm 0{\cdot}42$	Par.	$2{\cdot}58 \pm 0{\cdot}035$	$0{\cdot}37 \pm 0{\cdot}053$	—	2·3	E
	—	—	X	—	—	—	III	260 × 140	15	$y = 2{\cdot}45x - 0{\cdot}42$	$2{\cdot}45 \pm 0{\cdot}32$		$2{\cdot}21 \pm 0{\cdot}040$				
F	—	—	X	—	—	—	III	220 × 120	12	$y = 3{\cdot}68x - 3{\cdot}44$	$3{\cdot}68 \pm 0{\cdot}41$	Par.	$2{\cdot}29 \pm 0{\cdot}030$	$0{\cdot}15 \pm 0{\cdot}045$			F
	—	—	—	—	X	—	V	120	20	$y = 4{\cdot}01x - 3{\cdot}59$	$4{\cdot}01 \pm 0{\cdot}60$		$2{\cdot}14 \pm 0{\cdot}033$		0·12	1·3	
G	—	—	X	—	—	—	III	246 × 144	20	$y = 3{\cdot}05x - 1{\cdot}77$	$3{\cdot}05 \pm 0{\cdot}35$	Par.	$2{\cdot}22 \pm 0{\cdot}033$	$0{\cdot}08 \pm 0{\cdot}046$			G
	—	—	—	—	X	—	V	108	20	$y = 3{\cdot}01x - 1{\cdot}45$	$3{\cdot}01 \pm 0{\cdot}30$		$2{\cdot}14 \pm 0{\cdot}032$				
H	—	X	—	-	—	-	II	60 × 15	—	$y = 3{\cdot}79x - 4{\cdot}80$	$3{\cdot}79 \pm 0{\cdot}55$	Par.	$2{\cdot}59 \pm 0{\cdot}030$	$0{\cdot}12 \pm 0{\cdot}042$			H
	—	—	—	—	—	X	VI	35	20	$y = 3{\cdot}24x - 3{\cdot}00$	$3{\cdot}24 \pm 0{\cdot}41$		$2{\cdot}47 \pm 0{\cdot}030$		0·12	1·3	
I	—	X	—	—	—	—	II	60 × 15	—	$y = 4{\cdot}85x - 7{\cdot}12$	$4{\cdot}85 \pm 0{\cdot}63$	Par.	$2{\cdot}50 \pm 0{\cdot}023$	$0{\cdot}12 \pm 0{\cdot}030$			I
	—	—	—	—	-	X	VI	42	20	$y = 6{\cdot}10x - 9{\cdot}51$	$6{\cdot}10 \pm 0{\cdot}76$		$2{\cdot}38 \pm 0{\cdot}019$				
J	X	—	—	—	—	—	I	—	—	$y = 2{\cdot}08x - 0{\cdot}79$	$2{\cdot}08 \pm 0{\cdot}35$	Not par.	$2{\cdot}78 \pm 0{\cdot}046$	$0{\cdot}45 \pm 0{\cdot}051$			J
	—	X	—	—	—	—	II	60 × 15	—	$y = 4{\cdot}51x - 5{\cdot}51$	$4{\cdot}51 \pm 0{\cdot}53$		$2{\cdot}33 \pm 0{\cdot}023$		0·50	3·2	
K	X	—	—	—	—	—	I	—	—	$y = 2{\cdot}19x - 1{\cdot}43$	$2{\cdot}19 \pm 0{\cdot}34$	Par.	$2{\cdot}04 \pm 0{\cdot}052$	$0{\cdot}54 \pm 0{\cdot}059$			K
	—	X	—	—	—	—	II	60 × 15	—	$y = 3{\cdot}14x - 2{\cdot}55$	$3{\cdot}14 \pm 0{\cdot}39$		$2{\cdot}40 \pm 0{\cdot}029$				
L	X	—	—	—	—	—	I	—	—	$y = 1{\cdot}80x - 0{\cdot}31$	$1{\cdot}80 \pm 0{\cdot}21$	Not par.	$2{\cdot}95 \pm 0{\cdot}032$	$1{\cdot}33 \pm 0{\cdot}340$			L
	—	—	—	X	—	—	IV	—	—	$y = 5{\cdot}13x - 3{\cdot}30$	$5{\cdot}13 \pm 0{\cdot}32$		$1{\cdot}62 \pm 0{\cdot}012$		1·19	15·5	
M	X	—	—	—	—	—	I	—	—	$y = 1{\cdot}94x - 0{\cdot}43$	$1{\cdot}94 \pm 0{\cdot}34$	Not par.	$2{\cdot}80 \pm 0{\cdot}045$	$1{\cdot}05 \pm 0{\cdot}053$			M
	—	—	—	X	—	—	IV	—	—	$y = 3{\cdot}24x - 0{\cdot}66$	$3{\cdot}24 \pm 0{\cdot}43$		$1{\cdot}75 \pm 0{\cdot}029$				
N	II in 10 % acetone						—	60 × 15	—	$y = 4{\cdot}53x - 6{\cdot}54$	$4{\cdot}53 \pm 0{\cdot}52$	Par.	$2{\cdot}55 \pm 0{\cdot}022$	$0{\cdot}04 \pm 0{\cdot}032$	—	1·1	N
	II in 10 % alcohol						—	60 × 15	—	$y = 4{\cdot}31x - 5{\cdot}81$	$4{\cdot}31 \pm 0{\cdot}50$		$2{\cdot}51 \pm 0{\cdot}023$				
O	IV in 10 % acetone						—	—	—	$y = 4{\cdot}05x - 2{\cdot}23$	$4{\cdot}05 \pm 0{\cdot}53$	Par.	$1{\cdot}79 \pm 0{\cdot}025$	$0{\cdot}12 \pm 0{\cdot}042$	—	1·3	O
	IV in 10 % alcohol						—	—	—	$y = 3{\cdot}77x - 2{\cdot}20$	$3{\cdot}77 \pm 0{\cdot}57$		$1{\cdot}91 \pm 0{\cdot}033$				

All toxicity ratios are given with the concentration of the less toxic preparation as numerator.



Text-fig. 3. Graphs A–H. Results using the dipping technique.



Text-fig. 4. Graphs I–O. Results using the dipping technique. Graph P. Results using a modified dipping technique.

0% respectively. Some details of the probit analyses along with size measurements, where they were made, are given in Table 1. The marks '*X*' show the suspension types compared in the various tests. The equations take the form $y = bx + c$, where y = probit kill, b = gradient of line, x = log [percentage concentration $\times 10^4$] and c = constant. The values of x when $y = 5$ (i.e. log [median lethal concentration $\times 10^4$] or log [M.L.C.]) are calculated from the equations. The figures for 'mean ratio of M.L.C.' are the geometrical means of the individual ratios of median lethal concentrations. All the ratios are given with the concentration of the less toxic preparation as numerator.

It will be noted that the colloidal suspension always gave a flatter line than the other types. In the four tests involving the colloid, three of the pairs of lines are not parallel; in the eleven tests without the colloid, ten of the pairs of lines are parallel.

Tests N and O, which compare 10% acetone with 10% alcohol, show that the suspension containing acetone was more toxic in one test but less toxic in the other. It has therefore been assumed that alcohol and acetone can be interchanged without affecting the toxicity, and that quantities of solvent up to 10% do not influence the toxicity. Consequently we can directly compare the toxicities of short alcohol needles and colloidal suspensions (containing 10 and 7·5% alcohol respectively) with each other and with those of other suspension types containing 10% acetone.

Further, in view of the toxicity ratios in tests N and O, other pairs of suspensions which gave a ratio of between 1·0 and 1·3 when tested under like conditions must be regarded as of equal toxicity.

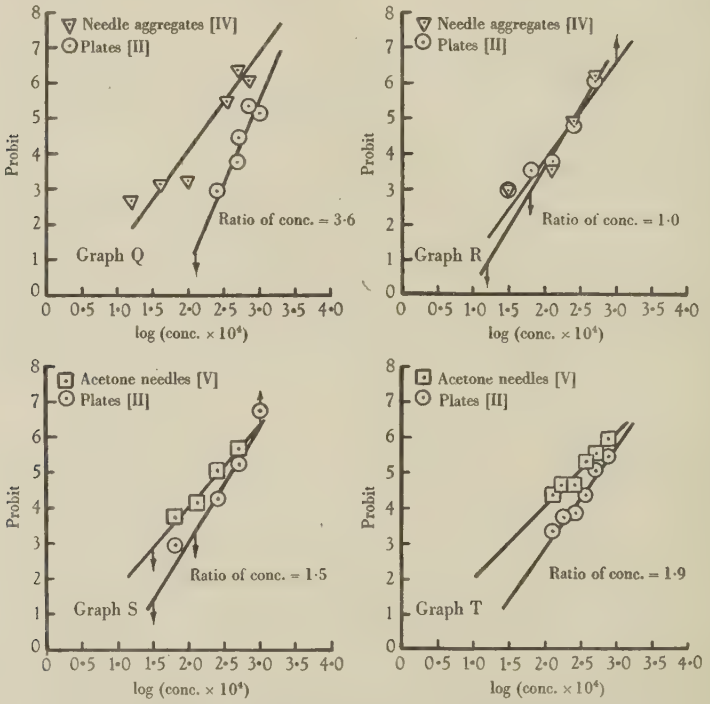
The figures show one irregularity. $60 \times 15\mu$ plates were less toxic than short acetone needles, the ratio of median lethal concentrations being 2·0 (test D). Also, $60 \times 15\mu$ plates were less toxic than plate aggregates, with a ratio of concentrations of 2·3 (test E). Thus it would be expected that plate aggregates would be slightly more toxic than short acetone needles. The reverse, however, was recorded. Two tests showed that the short acetone needles were the more toxic, the mean ratio of median lethal concentrations being 1·3 (tests F and G). However, if we disregard ratios of 1·3 and less, this difficulty disappears, and we can arrange the types in order of decreasing toxicity, thus: needle aggregates > short acetone needles = plate aggregates > short alcohol needles = $60 \times 15\mu$ plates > colloidal suspension.

From the results of tests A–M we can obtain, by the method of least squares, numerical values for the relative potencies of the six types. The standard errors shown in Table 1 are based on the internal evidence of each test. There is some heterogeneity between tests and for comparing them the standard errors of the values of log [M.L.C.] may be taken as double the values shown. The mean values for log [M.L.C.] for each type, taking that of the colloid as zero, are, for $60 \times 15\mu$ plates, $\bar{1}\cdot48$; for short alcohol needles, $\bar{1}\cdot36$; for plate aggregates, $\bar{1}\cdot18$; for short acetone needles, $\bar{1}\cdot09$; and for needle aggregates, $\bar{2}\cdot77$. From these values we can obtain the relative concentrations to which the logs correspond. If, for example, we take the M.L.C. for needle aggregates as 100, then the other values are, for short

acetone needles, 210; for plate aggregates, 260; for short alcohol needles, 390; for $60 \times 15\mu$ plates, 510; and for colloidal D.D.T., 1700.

Results using modifications of the dipping technique

When the suspension is drained from the insects at the end of a dipping period, the insects remain on the wet muslin even when the tube is inverted and placed in the constant-temperature room. In 1 or 2 hr. they fall down on to the clean muslin at the bottom of the tube.



Text-fig. 5. Graph Q. Results using a modified dipping technique. Graphs R, S, T. Results using the Potter spraying tower.

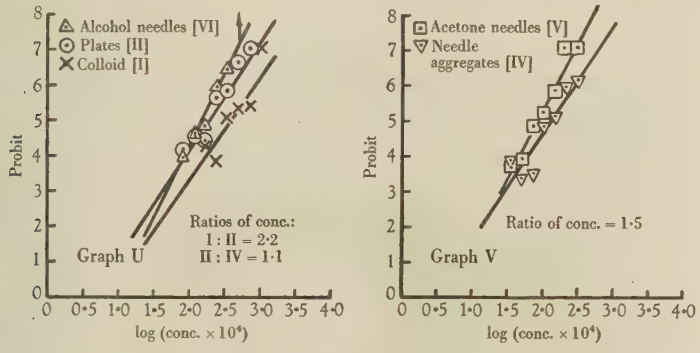
It was thought that an explanation of the above toxicity differences might be that, other things being equal, the muslin retains different amounts of D.D.T. from different suspension types. Tests showed, however, that while the D.D.T. that insects pick up from the muslin may contribute to the kill, the differences between suspension types persist when steps have been taken to eliminate any muslin adsorption effects.

If the muslin is tapped with the finger after it has been dried on filter-paper, the insects fall down on to the sides of the tube or on to the dry muslin at the bottom. Text-fig. 4, graph P, shows two lines obtained when this procedure was adopted,

using suspensions of 60 × 15 μ plates and plate aggregates. Table 2 shows details of the line.

These lines are nearly coincident with the pair obtained for the same suspensions in test E, which are shown as broken lines. Although the two tests were done at about the same time and with the same culture this must be regarded as fortuitous, for, as has been mentioned before, it is not usually possible to reproduce lines so exactly with different samples of suspension on different days. Here, also, the method of treatment is different. When the 'tapping-off' method is tested under equal conditions against the standard method, slightly lower kills are obtained. In the above example other variables have masked this effect. However, the concentration ratios obtained in this test and in test E are of the same order.

Results of a similar nature can be obtained using brass mesh instead of muslin to separate the insects from the suspension. A small square (2 × 2 in.) was held in



Text-fig. 6. Graphs U and V. Results using the Potter spraying tower.

contact with the mouth of the open dipping tube. Whilst the insects were still lying on the mesh, it was dried from the bottom on filter-paper, as with muslin, to remove excess suspension. The mesh was now inverted and the insects transferred to a clean Petri dish by a smart tap on the back of the mesh. They were kept at constant temperature in the usual way. Text-fig. 5, graph Q, shows some rather rough results obtained in this way, using needle aggregates and 60 × 15 μ plates. The lines were not calculated but estimated by eye for best fit. From the graph, the ratio of median lethal concentrations is found to be 3·6, which compares fairly well with the earlier value of 5·0 (tests A and B).

Results using the Potter spraying tower

The results of a few tests carried out using this apparatus are given in Table 3 and in Text-figs. 5 and 6, graphs R, S, T, U and V. With the finer suspensions the results are in fairly good agreement with those obtained by the dipping method; but with coarser crystals the toxicity ratios obtained by the two different methods are,

TABLE 2. *Results of test P*

Test	Suspension type: Colloid I	60 × 15 μ plates II	Plate aggregates III	Needle aggregates IV	Short acetone needles V	Short alcohol needles VI	Types tested	Mean size in μ	No. of samples for measurement	Equation	Standard error in b	Whether parallel	Log [M.L.C. × 10^4]	Diff. in logs	Mean diff. in logs	Mean ratio of M.L.C.	Test
P	—	X	—	—	—	—	II	60 × 15	—	$y = 2{\cdot}80x - 2{\cdot}36$	$2{\cdot}80 \pm 0{\cdot}40$	Par.	$2{\cdot}63 \pm 0{\cdot}040$	$0{\cdot}51 \pm 0{\cdot}054$	—	3·2	P
	—	—	X	—	—	—	III	210 × 140	12	$y = 2{\cdot}77x - 0{\cdot}87$	$2{\cdot}77 \pm 0{\cdot}35$		$2{\cdot}12 \pm 0{\cdot}036$				

TABLE 3. *Results of tests R–V*

Test	Suspension type: Colloid I	60 × 15 μ plates II	Plate aggregates III	Needle aggregates IV	Short acetone needles V	Short alcohol needles VI	Types tested	Mean size in μ	No. of samples for measurement	Equation	Standard error in b	Whether parallel	Log [M.L.C. × 10^4]	Diff. in logs	Mean diff. in logs	Mean ratio of M.L.C.	Test
R	—	X	—	—	—	—	II	60 × 15	—	$y = 2{\cdot}77x - 1{\cdot}72$	$2{\cdot}77 \pm 0{\cdot}34$	Par.	$2{\cdot}43 \pm 0{\cdot}041$	$0{\cdot}01 \pm 0{\cdot}055$	—	1·0	R
	—	—	—	X	—	—	IV	—	—	$y = 3{\cdot}28x - 2{\cdot}95$	$3{\cdot}28 \pm 0{\cdot}46$		$2{\cdot}42 \pm 0{\cdot}037$				
S	—	X	—	—	—	—	II	60 × 15	—	$y = 3{\cdot}32x - 3{\cdot}58$	$3{\cdot}32 \pm 0{\cdot}48$	Par.	$2{\cdot}58 \pm 0{\cdot}039$	$0{\cdot}17 \pm 0{\cdot}059$	0·23	1·7	S
	—	—	—	—	X	—	V	108	20	$y = 2{\cdot}31x - 0{\cdot}57$	$2{\cdot}31 \pm 0{\cdot}33$		$2{\cdot}41 \pm 0{\cdot}045$				
T	—	X	—	—	—	—	II	60 × 15	—	$y = 2{\cdot}83x - 2{\cdot}74$	$2{\cdot}83 \pm 0{\cdot}39$	Par.	$2{\cdot}73 \pm 0{\cdot}038$	$0{\cdot}28 \pm 0{\cdot}055$			T
	—	—	—	—	X	—	V	126	20	$y = 2{\cdot}02x + 0{\cdot}05$	$2{\cdot}02 \pm 0{\cdot}31$		$2{\cdot}45 \pm 0{\cdot}040$				
U	X	—	—	—	—	—	I	—	—	$y = 2{\cdot}90x - 2{\cdot}48$	$2{\cdot}90 \pm 0{\cdot}40$	All par.	$2{\cdot}58 \pm 0{\cdot}033$	$0{\cdot}34 \pm 0{\cdot}045$*	—	2·2	U
	—	X	—	—	—	—	II	60 × 15	—	$y = 3{\cdot}14x - 2{\cdot}04$	$3{\cdot}14 \pm 0{\cdot}36$		$2{\cdot}24 \pm 0{\cdot}030$	$0{\cdot}05 \pm 0{\cdot}039$†	—	1·1	
	—	—	—	—	—	X	VI	84	20	$y = 4{\cdot}02x - 3{\cdot}80$	$4{\cdot}02 \pm 0{\cdot}51$		$2{\cdot}19 \pm 0{\cdot}026$				
V	—	—	—	X	—	—	IV	—	—	$y = 3{\cdot}08x - 1{\cdot}45$	$3{\cdot}08 \pm 0{\cdot}38$	Par.	$2{\cdot}09 \pm 0{\cdot}030$	$0{\cdot}17 \pm 0{\cdot}038$	—	1·5	V
	—	—	—	—	X	—	V	—	—	$y = 3{\cdot}86x - 2{\cdot}41$	$3{\cdot}86 \pm 0{\cdot}38$		$1{\cdot}92 \pm 0{\cdot}023$				

All toxicity ratios are given with the concentration of the less toxic preparation as numerator.

* Type I–Type II. † Type II–Type VI.

TABLE 5. *Tests L and M (corrected for retention)*

Test	Suspension	Equations from Table 1	F	$a = x + \log F$	Equations of lines in Text-fig. 7, graphs X and Y	Log [M.L.C.]	Diff. in logs	Mean diff. in logs	Mean ratio of M.L.D.
L (X)	I	$y = 1{\cdot}80x - 0{\cdot}31$, i.e. $x = 0{\cdot}56y + 0{\cdot}17$	0·22	$a = x - 0{\cdot}66$	$a = 0{\cdot}56y - 0{\cdot}49$	2·31	0·30	0·15	1·4
	IV	$y = 5{\cdot}13x - 3{\cdot}30$, i.e. $x = 0{\cdot}19y + 0{\cdot}64$	2·62	$a = x + 0{\cdot}42$	$a = 0{\cdot}19y + 1{\cdot}06$	2·01			
M (Y)	I	$y = 1{\cdot}94x - 0{\cdot}43$, i.e. $x = 0{\cdot}52y + 0{\cdot}22$	0·22	$a = x - 0{\cdot}66$	$a = 0{\cdot}52y - 0{\cdot}44$	2·16	0·01		
	IV	$y = 3{\cdot}24x - 0{\cdot}66$, i.e. $x = 0{\cdot}31y + 0{\cdot}20$	2·62	$a = x + 0{\cdot}42$	$a = 0{\cdot}31y + 0{\cdot}62$	2·17			

Toxicity ratios are given with the weight of the less toxic preparation as numerator.

in some cases, conflicting. Thus, the results of tests D, H, I, J and K are in general agreement with those of tests S, T and U, although the ratios obtained in the later series are on the whole somewhat smaller than those obtained earlier. On the other hand, tests A and B showed the ratio of concentrations for $60 \times 15\mu$ plates and needle aggregates to be 5·0; test R showed the two types to be of equal toxicity. Test C showed that the needle aggregates are more toxic than short acetone needles, the ratio of concentrations being 2·1; the corresponding ratio from test V is 0·7, i.e. the order of toxicity is reversed.

It is easily shown that when the coarser suspensions (plate aggregates, needle aggregates, and short acetone needles) are used in the tower, the crystals are broken down into smaller units. This fragmentation is negligible or absent with the other three suspension types. Thus, measurements of mean crystal size of needle aggregates before passing through the nozzle gave values of 460 and 475μ in two cases; the corresponding figures for suspension collected at the base of the tower were 153 and 150μ. In the same way, short acetone needles were reduced from 146 to 78μ.

However, these facts do not by any means explain the very marked alterations recorded in the values of certain toxicity ratios on changing from one testing method to the other. The origin of the differences may be in varying distribution of poison on the Petri dish at the bottom of the tower, or in some other purely physical factor related to or arising from the fragmentation effect.

From the toxicity data obtained so far by the Potter spraying method, it appears that short acetone needles represent the optimum crystal size against *T. castaneum*; and where spraying does not reduce particle size, the results of the spraying and dipping tests are in agreement.

Origin of toxicity differences

Basis of comparison

The above toxicity differences might be explicable if it could be established that treatment with smaller crystals resulted in a greater 'run-off' of poison from the insects' bodies than treatment with larger ones (McIntosh, 1946). True comparison of the relative toxicities of different types *in situ* would be obtained by using the kills brought about by equal weights of poison retained by the insects after treatment and not by equal concentrations of poison in suspension. As D.D.T. is a slow-acting poison, it is the former quantity that would be expected to determine the kill. The D.D.T. retained by *T. castaneum* can be recovered by washing the insects with an organic solvent, and determined colorimetrically.

The experiments described below were carried out using benzene as the strip solvent and the analysis method of Schechter *et al.* (1945). The large number of insects required for each analysis necessitated the restriction of experiments to the two suspensions showing the greatest toxicity difference, viz. needle aggregates and colloidal D.D.T.

Methods

Method of dipping

The procedure was identical with the standard dipping technique except for the numbers of insects employed. For each analysis 750 insects were required. These were treated in eight batches, seven of 100 insects and one of fifty. The suspension was renewed after every two tubes instead of the three normally employed. The tubes were kept, as usual, at 27° C. between dipping and subsequent treatment. The analyses were carried out shortly after the insects were dry; a period of $4\frac{1}{2}$ hr. between dipping and washing was found convenient.

Stripping and analysis

Each set of 750 insects was treated as follows. The insects were collected, from the eight tubes, in a small conical flask, 15 ml. A.R. benzene were added and the liquid swirled gently for 10 min. at room temperature. The benzene was filtered into a dry boiling tube and the insects washed for 3 min. with each of two further 10 ml. portions of benzene, which were filtered. The combined filtrates were evaporated to dryness on a water-bath, with a jet of air blowing on the surface of the benzene. The last few drops were rather viscous and evaporated slowly. It was important to take the residue to complete dryness, as, unless evaporation was thorough, considerable interference occurred at the colour development stage.

To the dry residue was now added 5 ml. of 1:1 conc. HNO_3-conc. H_2SO_4 mixture, and the nitration and extraction carried out as described by Schechter *et al.* (1945). The final ether solution was evaporated to dryness in a boiling tube, which was kept in the refrigerator till the other analyses had reached the same stage.

Calibration points and control

Calibration points were obtained by washing 750 untreated insects in the manner described above and adding a suitable amount of a solution of D.D.T. in acetone containing 50 μg./ml. A blank for use in the colorimeter was obtained in a similar way.

In tests which did not involve dipping, the tedium of counting out the insects could be avoided by weighing; 750 *T. castaneum* weigh about 1·53 g. A calibration was carried out at the same time as each series of determinations.

Development and measurement of colour

The colorimeter used was a Spekker photoelectric instrument with 1 cm. cells, and with a 'Calorex' H503 and Ilford Spectrum Yellow filter on each side of the lamp.

To each of the residues in the boiling tubes was now added 5 ml. benzene; and the tubes were allowed to stand (stoppered) at room temperature for about half an hour with occasional gentle shaking to ensure complete dissolution. Then, to each in order, was added 10 ml. NaOMe solution, and the colorimeter reading for each taken after 15 min.

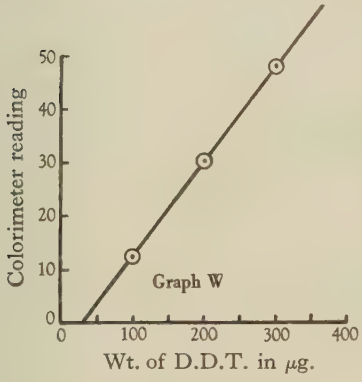
Results

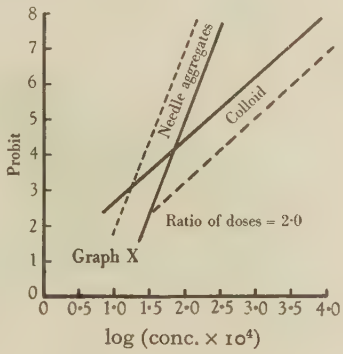
A typical calibration curve is shown in Text-fig. 7, graph W. Three points were used for each calibration curve, and on all occasions they lay on or very nearly on a straight line, which, when obtained in the above way with a Spekker instrument, did not usually pass through the origin.

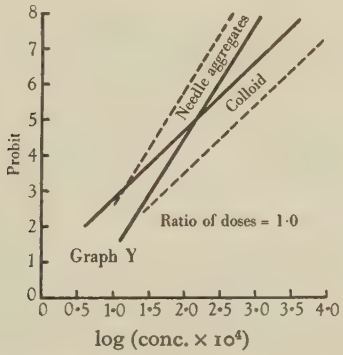
A summary of the amounts of D.D.T. retained by insects at various concentrations of needle aggregates and colloidal suspension is given in Table 4. The ratio (F) of the amount retained in micrograms (μg.) to concentration $\times 10^4$ (λ) is shown for each determination.

TABLE 4. *Summary of amounts of D.D.T. retained by insects*

Colloid				Needle aggregates			
λ	μg.	$F=\mu g./\lambda$	Mean F	λ	μg.	$F=\mu g./\lambda$	Mean F
200	71	0·36	0·22	25	56	2·24	2·62
400	75	0·19		60	216	3·60	
600	109	0·18		120	250	2·08	
1000	137	0·14		200	508	2·54	







Text-fig. 7. Graph W. Calibration curve. Graphs X and Y. Comparison of toxicities.

For each suspension type it is seen that there is considerable variation of F. However, as this is irregular it has been assumed that the factor F is in fact constant over a range of concentrations. The mean of the four values has been taken as this constant. Although this step is perhaps not really justifiable in view of the large variation in the values of F, its consequences are rather interesting.

If we now apply these figures to Text-fig. 4, graphs L and M, i.e. if we plot a ($=\log \mu g.$) instead of x ($=\log \lambda$) against probit, we obtain the new lines shown in Text-fig. 7, graphs X and Y, the broken lines being the original ones in Text-fig. 4, graphs L and M. The stages of the calculations involved are given in Table 5 (p. 604).

It is apparent that the ratio of toxicities of the two types, when compared in this way, has been much reduced; it has been reduced in fact to a value almost low enough for us to consider the two types as of identical toxicity.

Discussion

It seems reasonable to infer that the results obtained by the standard dipping method would be obtained by any other method for testing suspensions which applies the crystals to the insects unchanged in size and shape. The fact that certain results obtained by the spraying tower are the reverse of those from the dipping method demonstrates the great influence that the testing method exerts, and emphasizes the necessity for describing toxicity tests in detail.

Assuming that the results are general, we can arrange the suspension types in order of decreasing toxicity, thus:

IV	Needle aggregates (*c.* 400μ).	
V	Short acetone needles (*c.* 120μ)	identical toxicity.
III	Plate aggregates (*c.* 240 × 140μ)	
VI	Short alcohol needles (*c.* 40μ)	identical toxicity.
II	Plates (60 × 15μ)	
I	Colloidal suspension.	

Concerning the needles, it is obvious that, within the range of sizes tested, toxicity increases with length. Thus, needles of 400μ are more toxic than those of 120μ, and the 120μ than the 40μ. Moreover, the plate aggregates of 240μ have no greater potency than needles of 120μ, and plates of 60μ have the same toxicity as needles of 40μ. It would thus appear that toxicity is primarily correlated with overall crystal length, and that increasing breadth, if anything, subtracts from the potency, when comparisons are made in terms of equal concentrations of suspensions. It must be emphasized that these conclusions only apply to D.D.T. suspensions containing crystals of up to about 400μ and refer to tests on *T. castaneum* in which the crystals are not damaged in the course of application. It is, however, obvious that toxicity cannot continue to increase indefinitely with particle size, but whether these needle aggregates represent maximum toxicity is doubtful.

The above results are the reverse of what had been anticipated when the work was started, but are in agreement with those of Parkin & Green (1945), who found that the toxicity of residual D.D.T. films to houseflies increases with the age of the film and that this is related to slow crystallization of D.D.T. from a 'poorly toxic gum-like residue'.

It is hardly likely that a large crystal would of itself be more toxic than a number of smaller crystals of the same total weight applied to the same insect, for one would expect a more rapid and greater solubility (in cuticle waxes, etc.) of the small crystals than the large, giving a more rapid (or higher) kill. No time-mortality studies have been carried out but they are obviously desirable. It appears, however, from the later experiments, that the factor of foremost importance is the amount of D.D.T. retained by the insects. The indications are that the toxicity of the crystals *in situ* is independent of size, but the evidence for this view is weak. Further, it is perhaps unwise to generalize from a comparison of the colloidal and needle aggregate suspensions, especially as the colloid always gave a line with a slope differing from those given by the various crystalline suspensions. Owing to the large number of test subjects required, retention tests could not unfortunately be carried out on suspension types intermediate in crystal size between needle aggregates and colloid. It seems reasonable, nevertheless, to assume that the other types would have given intermediate values of F.

No evidence is available to explain why more poison should be retained from coarser than from finer suspensions. It may simply be that larger particles are more readily retained by irregularities on the cuticle surface. Were this true, similar results would be expected when other crystalline poisons are tested under the same conditions.

The suspensions used in the above experiments are of a simple type. Commercial suspensions, for field use, contain, in addition to D.D.T., large amounts of surface-active compounds, clay, etc. It would be interesting to determine how far the above results apply to such preparations. The nozzles of the spray jets used for applying these preparations are generally coarser than the nozzle of the Potter apparatus, and one would expect fewer complications from the 'breaking-up' effect.

McGovran, Cassil & Mayer (1940) found that, in the range 1·1–22μ, the amounts of Paris green retained by sprayed bean leaves increased with particle size of poison. Whether or not the coarser D.D.T. suspensions, simple or commercial, give a greater D.D.T. retention on sprayed surfaces such as walls or foliage, remains to be seen.

The author wishes to record his indebtedness to Dr F. Tattersfield for his general guidance; to Mr F. J. Anscombe for calculating the numerical values of the relative toxicities of the different suspension types, and for his advice and criticism on statistical matters; to Mr V. Stansfield for preparing the photomicrographs; and to the Ministry of Agriculture for grants towards the cost of the investigations.

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EXPLANATION OF PLATE 16

Fig. 1. 60 × 15 μ plates, × 45.
Fig. 2. Plate aggregates, × 45.
Fig. 3. Needle aggregates, × 45.
Fig. 4. Short acetone needles, × 45.
Fig. 5. Short alcohol needles, × 45.

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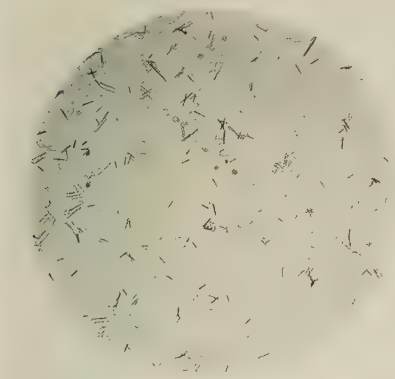

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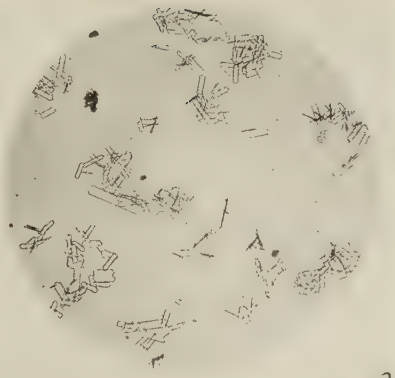

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McIntosh—*Particle size and toxicity of D.D.T. suspensions*

THE ECOLOGY OF THE BRITISH SPECIES OF *PSYCHODA* (DIPTERA: PSYCHODIDAE)

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(With 2 Text-figures)

A study of the dispersal of the sewage-filter Psychodids requires a knowledge of the breeding haunts and seasonal incidences of the local species of the genus. Field dung, decaying vegetable matter and organic mud form the breeding places to one of which some species confine themselves, whilst others, e.g. *Psychoda severini* Tonn., breed in a variety of materials. *P. alternata* Say is more restricted in its choice of breeding grounds and proved the best indicator of dispersal from sewage filters. With this fly, invasion of farms and woodland was apparent at a distance of $\frac{1}{4}$ and 1 mile respectively from the filters, but not at a distance of $1\frac{1}{2}$ miles in the direction of the prevailing wind.

Introduction

The genus *Psychoda* Latr. includes sixteen species and two subspecies in Britain (Tonnoir, 1940), and their larvae inhabit a variety of decaying organic materials such as dung, mud and rotting vegetation. Three species, *P. alternata* Say, *P. severini* Tonn. subsp. *parthenogenetica* Tonn. and *P. cinerea* Banks, have succeeded in colonizing the bacteria beds of sewage purification works, and *P. alternata* is so commonly present that it has become known as the trickling filter fly. *P. severini* is also frequently found in filter beds, but *P. cinerea* is less commonly present. These sewage-breeding species of *Psychoda* are sometimes so abundant that they become a household nuisance, especially when the sewage works are near towns (Lloyd, 1943), and Ordman (1946) has reported cases of bronchial asthma caused by inhaling the dust resulting from the disintegration of their bodies. No other specific association with disease has been reported for *Psychoda* flies, but insects from such highly contaminated environments must always come under suspicion.

No attempt at assessing the dispersal of the *Psychoda* flies from the sewage works can be made, however, until the abundance and seasonal incidence of the sewage-breeding species in the wild *Psychoda* fauna have been studied. Flies have, therefore, been collected both near to the sewage works and at such a distance away that dispersal from the works was unlikely to affect the fauna. As *Psychoda* flies are, apart from *P. alternata*, indistinguishable in the field, they must all be collected for identification in the laboratory. These collections have yielded information on the relative abundance of the non-sewage-breeding species of *Psychoda* and have shown that different species of *Psychoda* are common at different localities, depending on larval food supply. This latter point has also been studied by recording fly emergence from samples of likely breeding materials brought to the laboratory.

Materials and methods

In the identification of the species of *Psychoda* the descriptions of Tonnoir (1940) have been used, supplemented by those of Del Rosario (1936). The tip of the antenna is a feature of great diagnostic value, but it is readily damaged if the flies are roughly handled. They have, therefore, been collected by tubing them off trees and fences, and a definite area of ground has been worked over each week. This method of collecting is not fully adequate in periods of peak abundance, when it is virtually impossible to collect all the flies present.

To investigate the breeding places of the flies, media such as cow-dung, horse-dung, mud from mud flats and ditch bottoms, decaying grass and decaying leaves have been kept in the laboratory for a month to allow time for all the *Psychoda* flies to emerge. Longer retention led to the production of a second generation.

The collecting sites

Flies were collected by the author at three farms and three areas of woodland bordering on pasture, near Leeds. Collections were made weekly from October 1942 to July 1944. The collecting site at Meanwood Valley was a strip of woodland about 100 × 10 yd. lying between a pasture and a stream. This site lies in a public park, about $5\frac{1}{2}$ miles north-west of the Leeds sewage works at Knostrop. The site at Temple Newsam Park consisted of a row of trees with woodland on one side and pasture on the other, and included a stream and a small mud flat. This site was $1\frac{1}{2}$ miles east of the sewage works, in the direction of the prevailing wind. These two localities both proved to be sufficiently far from the sewage works to have a *Psychoda* fauna uninfluenced by dispersal of the sewage-bred flies, and they can be contrasted with the following four localities, all of which are nearer to the sewage works. Golf-Course Wood lay about $\frac{1}{4}$ mile due east of the sewage works. The collecting site was a row of trees where the wood bordered on a pasture. Adjoining Golf-Course Wood was farm A, where flies were collected in the outbuildings and dairy. Farm B lay south-west of the works just short of a mile away and flies were collected in the outbuildings. Farm C lay about a mile due west of the works and flies were collected from the outbuildings and around a silo.

The author is indebted to Dr Ll. Lloyd for data of other *Psychoda* collections taken at three localities all distant from the sewage works. Farm D, 4 miles north of the sewage works, provided a comparison with the three farms near the works. At Roundhay Park collections were made from trees surrounding a rubbish tip of kitchen waste in dense woodland, about $4\frac{1}{2}$ miles north-west of the sewage works. Lastly, Gledhow Valley, a site including a brook and a mud flat in which most of the sewage bacteria bed fauna occurred (Lloyd, 1944, 1945), lay about 4 miles north-west of the sewage works. The valley was wooded and there was pasture nearby.

To compare the composition of the *Psychoda* fauna at these localities the collections during each month were averaged and the resulting monthly indices for January to December 1943 were added together. The abundance of each species was then calculated as a percentage of the year's total.

The Psychoda *fauna of pasture land*

The *Psychoda* faunas of Meanwood Valley and Temple Newsam Park are alike (Table 1) and are recruited mainly from flies that breed in cow dung in the adjacent pastures. Table 2 gives a more detailed analysis of the fauna of Temple Newsam Park. Although slightly fewer flies were taken during 1943 at Meanwood Valley than at Temple Newsam Park, 859 against 1066, *P. phalaenoides* L. subsp. *elongata* Tonn. constituted just over half the total with 55% at Meanwood Valley and 61·4% at Temple Newsam Park. At both localities *P. setigera* Tonn. constituted just over 17%, whilst *P. albipennis* Zett. came third with 12·9 and 11·0% respectively. The remaining 10–15% was shared amongst the other nine species of the local fauna (Table 2). The three sewage-breeding species were extremely scarce and *P. alternata* was not taken at Meanwood Valley during 1943, and only once at

TABLE 1. *The percentage composition of the* Psychoda *fauna of nine localities near Leeds*

Locality	Total	*P. alternata*	*P. severini*	*P. cinerea*	*P. albipennis*	*P. phalaenoides*	Other species
Meanwood Valley	859	—	0·6	0·2	12·9	55·0	31·3
Temple Newsam Park	1066	0·1	2·0	0·4	11·0	61·4	25·1
Roundhay Park	451	—	14·9	1·9	33·1	33·6	16·5
Golf-Course Wood	329	29·9	20·8	0·2	12·3	12·5	24·3
Farm A	678	48·5	47·6	—	3·6	0·3	—
Farm B	653	3·7	95·2	—	1·0	—	0·1
Farm C	500	15·8	81·7	—	1·6	0·4	0·5
Farm D	1933	—	1·7	0·1	19·5	49·5	29·2
Gledhow Valley	1327	10·9	47·3	14·5	8·0	11·7	7·6

TABLE 2. *The monthly indices of the species of* Psychoda *taken at Temple Newsam Park during* 1943

	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Total	%
P. alternata	—	0·2	—	—	—	—	—	—	0·2	0·1
P. severini	1·0	0·7	1·0	0·8	1·0	—	0·2	0·2	4·9	2·0
P. albipennis	7·0	3·2	2·7	2·0	1·0	—	6·8	4·0	26·7	11·0
P. phalaenoides	—	2·5	29·5	78·8	12·0	5·0	4·0	16·6	148·4	61·4
P. setigera	4·0	9·7	11·0	7·6	1·2	7·0	1·5	—	42·0	17·4
P. brevicornis	—	—	0·7	—	—	0·5	0·3	—	1·5	0·6
P. cinerea	1·0	—	—	—	—	—	—	—	1·0	0·4
P. gemina	—	—	—	—	0·2	0·5	—	—	0·7	0·3
P. trinodulosa	—	—	1·2	2·4	0·5	4·0	0·3	—	8·4	3·4
P. grisescens	—	0·7	1·2	1·4	1·0	—	2·8	0·2	7·3	3·0
P. spreta	—	—	—	0·2	0·2	—	—	—	0·4	0·2
P. lobata	—	—	—	—	—	—	0·5	—	0·5	0·2
No. of collections	1	4	4	5	4	2	6	6		
No. of flies caught				1066						

Temple Newsam Park. *P. severini* constituted only 2% at Temple Newsam Park and 0·6% at Meanwood Valley, whilst *P. cinerea* was under 1% at both localities. Together the three sewage-breeding species accounted for just under 3% of the total flies.

At Golf-Course Wood (Table 3) only 329 flies were taken during 1943, and the influence of the adjacent sewage works was very apparent. *P. alternata* constituted 29·9% of the total and was the most abundant species, in marked contrast to the condition in the pasture-land fauna, where it was the scarcest (0·1%). *P. severini* was the second most abundant species, constituting 20·8% of the total. *P. albipennis* and *P. setigera* were approximately as common as in the pasture-land fauna (Table 2), whilst *P. phalaenoides* dropped to 12·5%. At Golf-Course Wood the fauna is thus seen to be largely dominated by flies dispersing from the sewage works. *P. cinerea* is scarce at the Golf-Course Wood because this species has not colonized the bacteria beds at Knostrop.

TABLE 3. *The monthly indices of the species of* Psychoda *taken at Golf-Course Wood during* 1943

	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Total	%
P. alternata	—	—	6·7	2·4	13·7	1·0	0·4	—	24·2	29·9
P. severini	1·0	2·2	5·0	2·2	4·2	—	1·6	0·6	16·8	20·8
P. albipennis	—	1·5	1·5	2·2	0·5	1·5	0·8	2·0	10·0	12·3
P. phalaenoides	—	0·7	1·0	5·4	0·7	0·5	0·2	1·7	10·2	12·5
P. setigera	—	4·0	5·7	1·2	0·2	0·5	0·2	—	11·8	14·6
P. brevicornis	—	—	—	—	0·2	—	—	—	0·2	0·2
P. cinerea	—	—	—	—	—	—	0·2	—	0·2	0·2
P. trinodulosa	—	—	0·2	—	—	—	—	—	0·2	0·2
P. grisescens	—	0·2	3·2	0·6	0·2	1·0	1·2	1·0	7·4	9·1
P. spreta	—	0·2	—	—	—	—	—	—	0·2	0·2
No. of collections	1	4	4	5	4	2	5	3		
No. of flies caught					329					

The Psychoda *fauna of farms*

The fauna of the three farms near the sewage works consisted almost entirely of sewage-breeding species (Table 1), but their abundance was not entirely due to dispersal from the sewage works. *P. alternata* breeds only in very wet and foul environments and is not a normal inhabitant of farms, but *P. severini* feeds on almost any moist decaying vegetable material. As there is a constant infiltration of adults from the sewage works to the farms any suitable breeding site may support a population of *P. severini* larvae. Consequently, the numbers of this species found on the farms is an index of their state of cleanliness rather than of their distance from the sewage works. Farm A (Table 1) showed approximately equal numbers of *P. severini* and *P. alternata*; it was within a $\frac{1}{4}$ mile of the sewage works, and dung was not left lying about the cow sheds to elevate the *P. severini* population. Farm C (Table 1) had many more *P. severini*, for, though this farm was equally close to the sewage works, this species colonized a layer of dung and straw left at one end of

a cow shed. Farm B (Table 1), nearly a mile away from the works, had *P. severini* present throughout the year. The species constituted 95·2% of the year's total, as a layer of dung and straw was left around the margins of a cow shed in which enormous numbers of the flies bred. It was observed that *P. severini* did not breed in stacked dung, but only in the material left in the sheds. At all these farms there was a large stack of dung, but no *P. severini* emerged from samples brought into the laboratory. On the other hand, dung collected from the floor margins of cow sheds at farm B produced numerous *P. severini*.

The fauna of these three farms may be compared with that of farm D, which was not only remote from the sewage works but free from conditions suitable for the breeding of *P. severini*. *P. alternata* was not taken at all during 1943 and *P. severini* constituted only 1·7% (Table 1). The fauna was very similar to the pasture-land fauna of Meanwood Valley and Temple Newsam Park, except that *P. albipennis* was rather more plentiful. The farm was surrounded by pasture containing numerous deposits of cow dung from which most of the farm *Psychoda* population evidently originated.

P. severini may be abundant at localities other than farms, and it constituted 14·9% of the total at Roundhay Park (Table 1). Here its prevalence was due to the suitable larval pabulum provided by a rubbish tip receiving kitchen waste. *P. albipennis* was also abundant at this locality (33·1%), and this species probably breeds in rubbish and decaying vegetation. Though it constitutes some 12% of the pasture-land fauna it has only twice emerged from field dung. It has, however, emerged from samples of decaying grass cuttings, rotten cabbages and rotten carrots. It was also very abundant (63·2%) in fourteen collections made by Dr Ll. Lloyd in his garden near Leeds in 1943, where its abundance was probably associated with a stack of rotting straw and manure.

In the Gledhow Valley mud flat many of the insects characteristic of the sewage bacteria bed occur (Lloyd, 1944, 1945). The *Psychoda* fauna, as judged by collections from the trees, consisted mostly of flies that had bred in the mud flat, plus some that had dispersed from the adjacent pasture land. *P. alternata* constituted 10·9%, *P. severini* 47·3% and *P. cinerea* 14·5% of the total, the remainder being pasture-land species (Table 1). Samples from the mud flat produced all three sewage-breeding species, and of 452 flies, *P. alternata* constituted 47·1%, *P. severini* 45·1% and *P. cinerea* 7·5%. The only non-sewage-breeding species was a single specimen of *P. grisescens* Tonn.

The seasonal incidence of Psychoda *species*

Fig. 1 illustrates the seasonal incidence of four species of *Psychoda* in the field. The two breeders in field dung, *P. phalaenoides* and *P. setigera*, each show a summer maximum followed by a depression, and the latter species shows a tendency to increase again in the autumn. The maximum follows the return of cattle to the pastures, but the reasons for the late summer depression and the autumn increase

are not evident. It may be an effect of competition due to the maximum activities of the higher Diptera in the dung deposit at this time. *P. albipennis*, breeding mainly in decayed vegetable matter, is most abundant in October when potential food is coming to its maximum, but before temperature has fallen seriously. *P. severini* shows a maximum in May and a steady decline towards autumn interrupted by a slight rise in August, at the Gledhow Valley mud flat. The abrupt rise in spring indicates a high winter survival, and the low-temperature threshold of larval development of this species, 0·6° C. (Lloyd, 1937), enables it to continue its

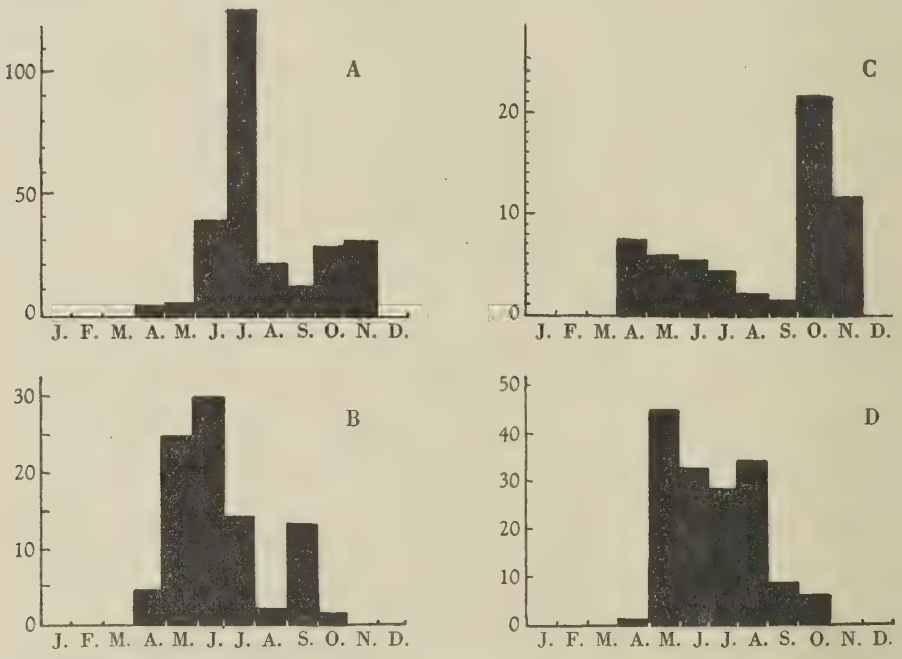

Fig. 1. The seasonal incidence of four species of *Psychoda*. A, *P. phalaenoides*; B, *P. setigera*; C, *P. albipennis*; D, *P. severini*. Ordinate: number of flies. Abscissa: months of the year.

development in all but the very coldest weather. At Gledhow its spring appearance is later than at the filter beds, where the warmth gives the species an early start; also it does not show the intense summer-autumn depression imposed on filter-bed *P. severini* by competition with Chironomids (Lloyd, 1937). This contrast between the natural and filter-bed seasonal incidence is also seen in *P. alternata* (Table 4), which increases steadily from spring to autumn at Gledhow, but at the filter bed exhibits a saddle-back curve of incidence, or a steady decline from an early summer maximum, due to the effects of competition (Lloyd, Graham & Reynoldson, 1940).

TABLE 4. *The monthly indices of the three sewage-breeding species of* Psychoda *at Gledhow Valley during* 1943

	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
P. alternata	—	—	1·7	2·0	2·0	9·5	9·7	10·8
P. severini	—	0·3	45·3	32·3	28·0	34·2	8·7	5·7
P. cinerea	0·5	—	1·7	2·3	4·0	13·5	5·3	19·8
No. of collections/month	2	3	3	3	4	4	6	6

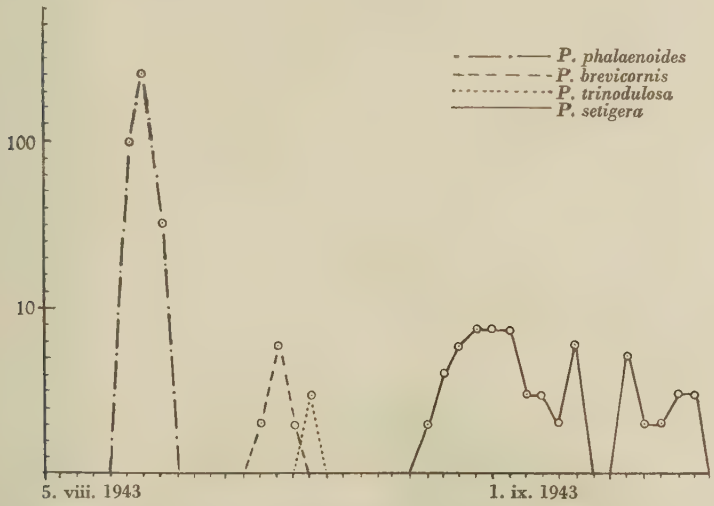

Fig. 2. Graph showing the emergence of *Psychoda* sp. from a sample of cow dung (collected 5 August 1943 near Leeds). Ordinate: log of number of flies (log $n+1$). Abscissa: time in days from date of collection.

The breeding media of Psychoda *flies and the succession of species in the field dung deposit*

By recording the fly emergence from eighty-nine samples of cow dung it was found that eight of the fourteen species of *Psychoda* taken in the field breed in this medium. The species emerge in a definite order, though not every sample of dung had all the members of the succession in it, and many samples were collected after the earlier emerging species had gone. This succession is well shown by a sample collected 5 August 1943 at Temple Newsam Park (Fig. 2). To elucidate this succession, the length of life cycle of a number of species was ascertained by setting up fertile females on scalded dung. The figures given refer to the time elapsing between oviposition and the emergence of the adults, and does not include the time taken for the maturation of the female, as a number of species would not mate in the laboratory.

In the twenty samples of cow dung from which *P. phalaenoides* was recorded this species was always the first to emerge. It has the shortest life cycle of any of the

species tested, 8 days at 20° C. Of the fourteen samples from which *P. grisescens* appeared, it emerged after *P. phalaenoides* on thirteen occasions and with it on one. Its length of life cycle at 20° C. is 12 days, which would account for its relegation to second position in the succession. *P. grisescens* is followed by two species, *P. brevicornis* Tonn. and *P. trinodulosa* Tonn., but it has not proved possible to ascertain the length of the life cycle of these species owing to the shortage of fertile females in the collections. Following these, *P. setigera* emerges; it has a life cycle of 21 days at 20° C. *P. albipennis* does not normally occur in the dung deposit, but on the two occasions when it did emerge, it was once just before *P. setigera* and once just after. Its life cycle at 20° C. takes 19 days.

In addition to the six species of *Psychoda* already mentioned as breeding in cow dung, *P. surcoufi* Tonn. and *P. crassipenis* Tonn. are known to breed in this medium. *P. surcoufi* emerged from two samples collected during November 1943, though only a single specimen of this fly was ever taken in the collections. Its length of life cycle at 20° C. is 15 days. *P. crassipenis* was found by Dr Lloyd breeding in a sample of cow dung collected at Appleby in September 1943, but the species has not emerged from this medium collected near Leeds.

There remain six species of *Psychoda* taken in the collections which do not normally breed in the field dung deposit, and the records of their emergences from various media have been dealt with in a previous paper (Satchell, 1947). *P. severini* does not often emerge from field deposits of cow dung, and of the eighty-nine samples collected, it was present in only two. It does, however, breed in decaying vegetable materials and has emerged from horse and chicken dung, decaying leaves, cabbage stalks, carrots, and mangolds, the slime adhering to the sides of drains, mud from mud flats, and the bacteria beds of sewage works. *P. alternata* has never emerged from the samples of field dung. It tends to be restricted to foul and wet environments such as privies, urinals, drains, rotting straw and dung in disused feeding troughs, carrion, mud flats and bacteria beds. *P. cinerea* has also never emerged from samples of field dung, and has been recorded from drains, a water trough containing algae, mud flats and bacteria beds.

P. gemina Eaton has twice emerged from samples of mud and leaves from a ditch, and *P. spreta* Tonn. appeared in a sample of rotting lawn mowings exposed at Meanwood Valley for a week. *P. lobata* Tonn., a scarce species around Leeds, has never emerged from any of the samples of material collected, and its larval food is unknown.

Parasites of Psychoda *flies*

Whilst examining the various species of *Psychoda* a number of parasites have been found. Bovien (1937) observed that *Psychoda* flies commonly carry the larval stages of species of *Rhabdites*. The worms wrap themselves tightly round the abdomen of the adult in the grooves between the segments, and were frequently found on flies collected in the field, and on specimens emerging from dung samples. The species most commonly infected was *Psychoda phalaenoides*, the only other infected species

found being *P. grisescens*. In the laboratory any species will pick up the worms if enclosed over a dung sample containing them. One culture was examined by Dr Goodey who identified it as *Rhabdites curvicaudata* Schneider (Goodey, 1943).

An internal parasitic nematode which almost fills the body cavity with its eggs and larvae was encountered on five occasions, four times in *Psychoda grisescens* and once in *P. spreta*. Dr Goodey kindly inspected a preparation and gave his opinion that this worm is allied to *Tylenchinema oscinella* Goodey (Goodey, 1930).

A protozoan parasite, a species of *Glaucoma*, probably *G. piriformis*, was encountered as a massive infection of the body cavity in five flies, three times in *Psychoda severini* and once each in *P. gemina* and *P. cinerea*. Watson (1946) has surveyed the versatile habits of *Glaucoma piriformis*, noting it as polysaprobic, coprophilic, and a potential parasite of invertebrates and cold-blooded vertebrates. In addition to these records in *Psychoda*, Lloyd (unpublished) has observed it in the Chironomid *Spaniotoma minima* Meig. from the filters.

Discussion

The *Psychoda* fauna of pasture land, exemplified by that of Meanwood Valley and Temple Newsam Park, was shown to be dominated by *P. phalaenoides*, a species that has frequently emerged from samples of field dung, but never from any other material. The totals for the three other exclusively dung-breeding species, *P. setigera*, *P. trinodulosa* and *P. brevicornis*, when added to that of *P. phalaenoides*, are found to account for over 80% of the pasture-land fauna. The only species of any numerical importance that is not usually a dung breeder is *P. albipennis*.

Of the few sewage-breeding *Psychoda* in the pasture-land fauna, *P. severini* is the most abundant, constituting 2% at Temple Newsam Park, and when suitable breeding sites are available it may be common. At Roundhay Park, a rubbish tip containing kitchen waste provided it with suitable larval food and it constituted 14·9% of the total. The abundance of this species at the three farms near the sewage works was in part due to dispersal from the filter beds and in part to fly breeding in the cow-shed litter. *P. severini* is probably a common farm insect, passing unnoticed because of its small size. It occurred at ten other farms visited during 1943 (five in Cumberland, three in Yorkshire, two in Shropshire), but is not mentioned by Thomsen & Hammer (1936) in their study of farm-breeding flies in Denmark.

Though *P. severini* is thus seen to be a common species which may be present wherever damp and decaying vegetable material abounds, *P. alternata* is much more restricted in its breeding sites, and consequently in its occurrence. It was common at Gledhow Valley where samples of mud showed it to be breeding in the mud flat, and at Golf-Course Wood, farm A and farm C. Here, however, its presence was due to dispersal, for not only were the bacteria beds within $\frac{1}{4}$ mile, but other suitable breeding grounds were absent. Further, it was observed that peak emergencies of *P. alternata* at the bacteria beds were followed by the appearance of

the species in abundance at the farms and at Golf-Course Wood. The abundance of this species at the farms reflects their distance from the sewage works, farm D, 4 miles away, showing none, farm B, 1 mile away, showing 3·7%, farm C, ¼ mile west, showing 15·8% and farm A, ¼ mile east, showing 48·5%. This species has, however, but feeble powers of migration, for at Temple Newsam Park, only 1½ miles away, only a single specimen was taken during almost 2 years' collecting. It is impossible to determine the dispersal powers of *P. severini*, for odd stragglers may colonize some naturally occurring habitat near the works and provide a new focus of abundance of the species, and the wide variety of vegetable matter that will serve this species as food makes it unsuitable as an index of sewage fly dispersal. A series of naturally occurring breeding sites at increasing distances from the sewage works may give the appearance of a very widespread dispersal when, in fact, the distance has been covered in stages.

Since the warmth of the beds during the winter promotes early emergence, a sewage works may be indirectly responsible for a severe fly nuisance even when the flies in question have not emerged from the works but from some naturally occurring breeding site. The early production of a species from the bacteria bed ensures a supply of fertile females ready to oviposit in any naturally occurring habitat, and this early start in the outside breeding foci may result in the species reaching troublesome proportions later in the year. This does not apply so much to species like *P. alternata* and *P. cinerea* which are restricted to wet and foul environments of a rather specialized type, for such species will be less able to find outside breeding foci of a suitable type within the dispersal range of the species from the sewage works. It does apply, however, to species like *P. severini* which is not so restricted. It is thus of importance to the sewage works operator to know, when complaints of sewage fly dispersal are received, not only the species of fly concerned, but also its natural breeding habitats and the extent to which such habitats are likely to be present within the dispersal range of the sewage works.

It is a pleasure to acknowledge my indebtedness to Dr Ll. Lloyd, both for permission to draw upon his data and for his constant help and advice. Thanks are also due to Prof. E. A. Spaul for discussion in the course of this work, and to the Department of Scientific and Industrial Research who provided a grant which enabled this study to be made.

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(*Received* 17 *April* 1947)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 3 October 1947, in the Imperial College of Science and Technology, London; the President, Mr W. C. Moore, in the Chair.

Problems associated with the potato.

The following papers were read and discussed:

1. Some observations on South American potatoes. By Dr J. G. Hawkes.
2. Blight in relation to potato breeding. By Dr W. Black.
3. Potato storage in North America. By Dr A. R. Wilson.*
4. A survey of Scottish seed tuber problems. By Dr C. E. Foister.
5. Some recent results of potato dry rot research. By Dr A. E. W. Boyd.

SOME OBSERVATIONS ON SOUTH AMERICAN POTATOES

By J. G. Hawkes, *School of Agriculture, Cambridge*

(With 4 Text-figures)

The importance of the potato crop at the present day is so obvious that it hardly needs stressing. The average total world crop for 1935–9 amounted to some 143 million tons grown on something more than 52 million acres. The potato has its pests and diseases which tend to reduce its yield, and it does best in a strictly limited habitat. In this paper I wish to deal, from the plant-breeder's point of view, with the attempts that have been made to solve some of the problems of disease attack and adaptation to new environments. And I further wish to restrict myself to the indigenous potatoes of the American continent, generally referred to as South American potatoes.

In attempting to solve the problem of disease resistance in any particular crop one can first use varieties that possess partial resistance and by appropriate selection attempt to build up that resistance to the required strength during the course of many generations. Secondly, one can search among wild relatives or allied species for plants completely immune to the disease in question and then attempt to introduce the gene or genes for resistance into high-yielding cultivated varieties. It is this latter method that is used to a great extent now, not only in potatoes but also in other crops. Thus the plant breeder, to solve his problems, must call in the systematist to identify the wild species with which he is dealing; the cytologist to count their chromosomes, indicate what success may be expected in species crosses and investigate the relationship of species hybrids; and the geneticist to obtain information on the methods of inheritance of the resistance obtained. Then, naturally, the plant pathologist and physiologist will be called in to deal with special problems in the diseases that attack the potato. Furthermore, investigations on the relationships and origins of the species from a taxonomic and cytogenetic point of view raise problems that may be solved only with the

* See *Report of the potato storage mission to the United States and Canada.* A.R.C. Rept. ser. 6. London: H.M. Stationery Office, 1947.

help of the archaeologist and ethnologist. This has been seen with such well investigated crops as wheat, maize and cotton; and with the potato similar methods of co-ordinating the results of specialists of many different types are now being used.

Modern potato breeding dated from the first decade of this century when Dr R. N. Salaman discovered true blight immunity in a wild potato species from Mexico. Several countries sent large expeditions to the Americas in the late twenties and early thirties to obtain wild potatoes for breeding, chief in size and scope being that sent by the U.S.S.R. In 1939, on the initiative of Dr P. S. Hudson, an expedition was sent by the Imperial Agricultural Bureaux that exceeded any of the previous ones in amount of potato material collected. This expedition made what is now known as the Empire Potato Collection, which is housed in Cambridge at the Empire Potato Station. The route taken by the expedition lay through most of the main potato growing districts of South America, in Colombia, Ecuador, Peru, Bolivia and North Argentina. Collections were chiefly made in the Andes at heights of up to 14,000 feet, but samples were also taken right down to sea level. Over 1000 lines were brought back, and the collection forms the basis of an inter-empire scheme for investigation in potato breeding. It is financed through the Imperial Agricultural Bureaux from funds provided from Dominion and Colonial governments. At the time of writing there are some 1500 lines maintained in the collection. The sorting out and testing of the various lines is centralized in Cambridge and work is now in progress on their taxonomy, cytology, virus content and resistance, late blight resistance, frost resistance, chemical composition and many other aspects. Material is distributed to Empire countries and to potato breeders in Great Britain for working up into new varieties suitable for the local conditions of the countries concerned. Work at Cambridge is confined to investigations of the properties of the potatoes and studying methods of transmission of these properties from parent to offspring.

After this brief outline of the work in progress at Cambridge I wish to continue with an account of the indigenous species of American potatoes and the investigations that have been carried out on them at Cambridge and elsewhere. I shall deal chiefly with the taxonomy, distribution, and cytology, hoping to show how a study along these particular lines is helping to solve problems in potato breeding in conjunction with the other work on resistance to diseases and pests.

The indigenous American potatoes occur both wild and truly cultivated, and it was samples of these cultivated potatoes that were brought over to Europe in the latter part of the sixteenth century. Wild potatoes, as can be seen in map 1 (Fig. 2), occur from the United States southwards through Mexico, Central America and South America, though in this latter sub-continent they are chiefly confined to the high lands of the Andes and the more temperate plains of Argentina. They are not found to any extent in the tropical Amazonian forests.

In North and South America, potatoes occur in nearly all the main phyto-geographic regions with the exception of tropical grassland, tropical forest and very high Andean ice-desert.

Many wild potatoes occur in the semi-deserts of North Argentina and South Bolivia and from their xeromorphic habit might be of value in breeding for drought resistance. Others occur in wet Andean sub-tropical forest where the rainfall is very high. It is clear that the wild potatoes occur in a very wide habitat range, far wider than our cultivated potato of Europe is able to withstand; and a study of the distribution of the wild potato is obviously of value to the breeder looking for initial material for breeding for resistance to extremes of heat, cold, drought, etc.

The indigenous cultivated potato is more restricted in its range since it is to be found (see maps 2 and 3, Fig. 3) only in temperate to cool temperate regions in the Andes, in South Chile and possibly also in Mexico and Central America. The greatest concentration of species occurs in Peru and Bolivia and it is from this and other facts that we are led to suppose that this region was the centre of origin of the cultivated potato. The civilizations here, both in the

coast and mountains, are the oldest known for South America; and it is fairly well substantiated that the potato was under cultivation at quite an early stage, as many samples of the ceramics of the ancient Indians indicate.

To return now to the classification of the potato. The genus *Solanum*, to which all potatoes belong, is very large, comprising some 2000 species; but only about 200 of these, at a generous estimate, are tuber-bearing. These belong to the subsection *Tuberarium* and are characterized chiefly by the simple anthers, absence of spines or thorns, and the articulated flower-stalk or pedicel. *Tuberarium* is further subdivided (see Fig. 1) according to whether the pedicel is articulated at the base (*Basarthrum*)—all the species in this division being non-tuberiferous—or

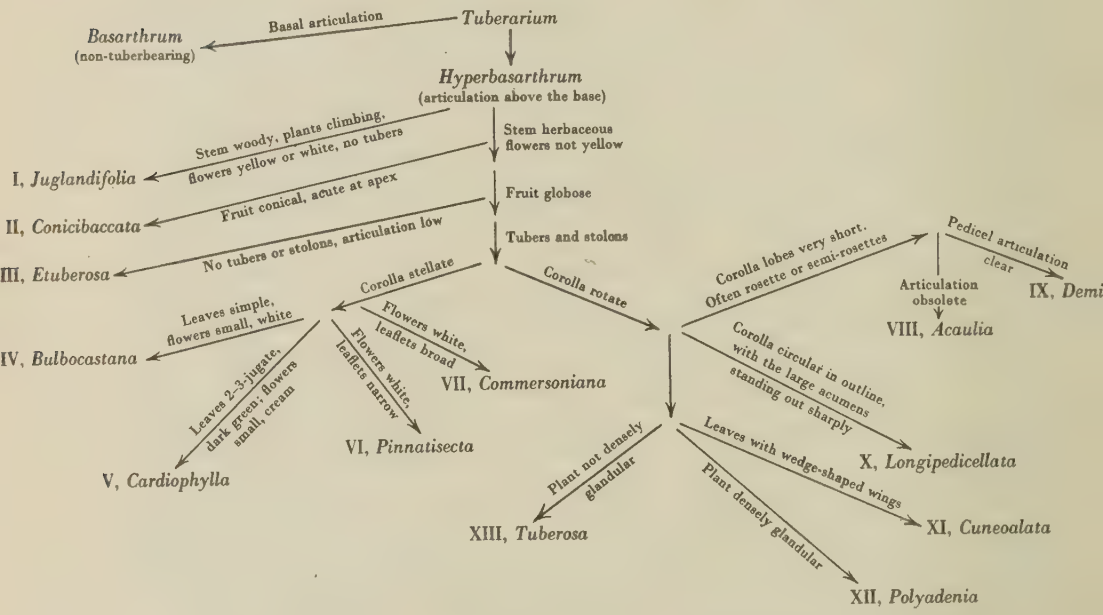

Fig. 1. Classification of potato species groups.

some way above the base (*Hyperbasarthrum*). *Hyperbasarthrum*, which contains all the tuber-bearing species, is further divided into the following series:

I, *Juglandifolia* distinguished from the other species by the woody stem, climbing or scrambling habit and yellow or white flowers. No species in this group bears tubers.

The herbaceous plants are further divided on berry shape, those with conical berries, generally more or less acute at the apex, going in to series II, *Conicibaccata*. The globose (or semi-cordate) berried species are next classified on presence or absence of tubers. If absent, and if the plants develop no stolons and possess an extremely low pedicel articulation they are classed into series III, *Etuberosa*. The remainder of the species with stolons and tubers are grouped on corolla shape, which can be either stellate or rotate. There are four series with stellate corollas:

IV, *Bulbocastana* with very small flowers and simple leaves;

V, *Cardiophylla* with medium small flowers, of a cream colour, and leaves dark green and shining, 2–3 jugate;

Fig. 2, map. 1. Geographical distribution of wild potato species in North and South America. The thirteen series or groups of species are indicated by different types of shading. The distribution of V, *Cardiophylla*, IX, *Demissa* and X, *Longipedicellata* in Central Mexico is, however, so nearly coincident that only one type of shading is given for all three.*

* Since this map was prepared the Mexican and Southern U.S.A. species *S. Fendleri*, formerly grouped with *Tuberosa*, has been transferred to *Longipedicellata*. The distribution line for *Tuberosa* in these regions should therefore now refer to *Longipedicellata*.

Map 2. Distribution of diploid and pentaploid cultivated potatoes in South America.

——— *Solanum stenotomum* (2*n*=24); — — — *S. Yabari* (2*n*=24); ⊕ *S. Churuspi* (2*n*=24); ····· *S. goniocalyx* (2*n*=24); ● *S. Cardenasii* (2*n*=24); ooooo *S. Phureja* (2*n*=24); -·-·-·- *S. Ajanhuiri* (2*n*=24); ◍ *S. Ascasabii* (2*n*=24); o-o-o-o *S. Kesselbrenneri* (2*n*=24); ++++ *S. Rybinii* (2*n*=24); ||||||||| *S. curtilobum* (2*n*=60).

Map 3. Distribution of triploid and tetraploid cultivated potatoes in South America.

++++ *S. Juzepczukii* (2*n*=36); o-o-o-o *S. Chauch* (2*n*=36); ····· *S. tenuifilamentum* (2*n*=36); ● *S. mamilliferum* (2*n*=36); ◍ *S. coeruleiflorum* (2*n*=36); -·-·-·- 3x hybrids (2*n*=36); ——— *S. andigenum* (2*n*=48); ◍ *S. tuberosum* [according to Dr S. M. Bukasov] (2*n*=48).

Fig. 3.

(Maps 1, 2, 3 reproduced by kind permission of the Clarendon Press from the *Empire Journal of Experimental Agriculture*, 13, 20–21 (1945).)

VI, *Pinnatisecta* with large white flowers and fairly narrow leaflets, the leaf itself being pinnatisect rather than pinnate;

VII, *Commersoniana*, with very similar flowers, white and large, but with broader leaflets, the leaf itself being quite definitely pinnate.

The first three of these stellate-corolla series are from the north American continent, the fourth from South America, and none seem to be very closely related, judging from attempts at hybridization.

The rotate corolla species possess two groups in which the acumen of the flower is very small and insignificant in relation to the lobe which is well developed. These are high-altitude rosette or semi-rosette species, and are divided on the basis of the pedicel articulation. In VIII, *Acaulia* the articulation is obsolete, or almost so, whilst in IX, *Demissa* it is well-marked. But in both series it is very high up under the calyx. Those species that do not fit into the previous series, that is to say, those with rotate corolla and the lobes not excessively flattened are then classified as follows: One series, X, *Longipedicellata*, possesses longish pedicels and corollas that are circular in outline, with the large triangular lobes standing out sharply. Another series, XI, *Cuneoalata*, possesses plants of small size with pinnatifid leaves, the midrib bearing very characteristic wedge-shaped wings. Another again, XII, *Polyadenia*, possessing only one species, *S. polyadenium*, is characterized by dense glandular pubescence and does not seem to be closely related to any other group. Finally, what is left after all these groups of species have been whittled away is a somewhat heterogeneous set of species grouped under XIII, *Tuberosa*. The cultivated potatoes are all classified into this series, together with their nearest wild and weed relatives. Here are also included species insufficiently studied as yet for us to be able to determine their exact affinities.

The geographical distribution of the species groups in the American continent (map 1, Fig. 2) is as follows:

I, *Juglandifolia*: Colombia, Ecuador, North Peru.

These plants have not been studied in the living state, but they would seem to be of no economic importance.

II, *Conicibaccata*: Mexico, Central America, Venezuela, Colombia, Ecuador, Peru, Bolivia.

Probably all are tuberiferous and some have been studied experimentally; none, however, seems to be of value to the plant breeder so far. They come from rather wetter, hotter regions than the majority of wild potato species and they might be of value in breeding for lower, more humid altitudes. Unfortunately we have not yet been able to cross them with anything else.

III, *Etuberosa*: South Central Chile, also in Argentina just over the Chilean border.

These plants bear no tubers and stolons, nor has it been possible to induce stolon formation by grafting tuberiferous species on to them. The one species in this series studied at Cambridge, *S. brevidens*, has been found by Dr W. R. Wortley to be apparently immune to all the usual sap-transmissible potato viruses and hence would be of great value if we could cross it with any tuber-bearing species.

IV, *Bulbocastana*: Mexico, Guatemala (? and Colombia).

The one species in this series so far studied is blight immune* but will not hybridize with anything else.

V, *Cardiophylla*: Mexico.

Here again we get blight immunity, but so far no hybrids between these species and other series.

VI, *Pinnatisecta*: Southern U.S.A., Mexico, Guatemala.

S. Jamesii, studied in the living state, is resistant to Colorado beetle but so far has resisted crossing with all other species, though none in its own series has yet been tried.

* All information in this paper on resistance to blight is based on tests made by Mr M. Petterson and Dr S. Dickinson.

VII, *Commersoniana*: Argentina, Uruguay, Paraguay, South Brazil, South Bolivia.

Resistance to Colorado beetle and frost has been found in this series, and most species will cross with *Tuberosa*, thus indicating its close affinities to this latter series. *S. chacoense* (*sensu latiore*) has been chiefly used in breeding for Colorado beetle resistance.

VIII, *Acaulia*: Peru, Bolivia, Northern Argentina.

These are all frost-resistant, very high altitude species and will cross with cultivated potatoes. They have been used quite considerably in breeding for frost resistance.

IX, *Demissa*: Mexico.

This is the Mexican phyto-geographical equivalent of *Acaulia*, the species however not being such typical rosette-formers, and characterized by a high degree of resistance to blight, resistance to frost and to Colorado beetle. *S. demissum* and *S. verrucosum* have been chiefly used, especially *S. demissum*, in breeding for blight resistance, and Drs Cockerham and Wortley have found several varieties of great promise in respect of virus resistance.

X, *Longipedicellata*: Mexico, Southern U.S.A.

All these species will hybridize with *Tuberosa* and most possess blight resistance, though not true immunity. Virus resistance is also to be found in some lines.

XI, *Cuneoalata*: this is a small series from the desert regions of South Bolivia, Northern Argentina and Northern Chile.

Little work has been done on the species in this series, and, though it is possible that they may be drought resistant, they have not yet been hybridized with anything else.

XII, *Polyadenia*: Mexico.

Blight and Colorado beetle immune. Again, no hybrids have been formed with any other species.

XIII, *Tuberosa*: this series is confined to South America, almost entirely to the Andes and their immediate surroundings.

Amongst the wild species some may be of value, such as *S. simplicifolium* for virus resistance, and some, as *S. Bukasovii* and *S. Abbottianum* are reported to be moderately resistant to frost ($-3°$). A species of promise is the newly described *S. Ballsii* from Northern Argentina which so far has resisted all attempts by Dr C. Ellenby to induce the potato root eelworm (*Heterodera rostochiensis*) to form cysts on its roots.

I have not spoken before of chromosome numbers since I shall return to that subject later. Here, however, I must state, in connexion with the cultivated species of potatoes (see maps 2 and 3, Fig 3), that a polyploid series occurs, with 12 as the basic number. We find, diploid ($2n=24$), triploid ($2n=36$), tetraploid ($2n=48$) and pentaploid ($2n=60$) cultivated species, and in the wild species all these with one hexaploid ($2n=72$) in addition. The diploid cultivated potatoes occur in greatest number but each species possesses a more restricted range. The tetraploid and pentaploid species, on the other hand, are fewer in number, but their geographical range is greater. The triploids occur in the area of greatest concentration of species where the diploids and tetraploids overlap. With one exception they are thought to have been formed as natural hybrids between diploid and tetraploid cultivated species. The exception is *S. Juzepczukii*, which is generally supposed to have been produced naturally by a cross between the wild frost-resistant species *S. acaule* and a variety of the cultivated diploid *S. stenotomum*, and I have been able to recreate *S. Juzepczukii* artificially by making this cross. Characters of economic value in these primitive cultivated species are as follows:

(1) *Frost resistance* found in *S. Juzepczukii*, *S. curtilobum*, *S. Ajanhuiri* and some clones of *S. stenotomum*. (Conclusion based partly on unpublished data from Mr C. M. Driver.)

(2) *Earliness, growth at higher temperatures and short dormancy: S. Phureja*, *S. Cardenasii*, *S. Rybinii*, *S. Kesselbrenneri* and *S. Ascasabii*.

(3) *High yield: S. andigenum*, *S. tuberosum* (now known as *S. tuberosum* subsp. *andigenum* and *S. tuberosum* subsp. *chileanum*), but the factors for high yield in South American potatoes, despite the claims of some Russian breeders, are probably no greater than we already possess in domestic potato varieties.

On the whole, when breeding for disease resistance, and for definite characters that are completely unknown in the domestic potatoes, the indigenous cultivated potatoes are of less value than the truly wild ones.

I want now to return to a more detailed consideration of chromosome number as throwing some light on the relationships of the species and groups.

On reference to Table 1 it will be seen that the groups or series have been arranged according to whether they occur in Mexico or in South America. Only one series, *Conicibaccata*, is to be found in both sub-continents. The species of this latter series are to be found in Central America, also, and as they are adapted to lower altitudes and rain-forest vegetation zones they have presumably been able to pass over the isthmus joining the two sub-continents. There are indications that *S. bulbocastanum* (in series IV, *Bulbocastana*) has also spread as far south as Colombia from its region of greatest abundance, which is Mexico. But apart from these two exceptions the series are found in either but not both of the sub-continents.

Table 1. *Correlation between species groups and chromosome numbers*

(*a*) *Mexico*	Chromosome numbers				
Conicibaccata	—	—	48	—	—
Bulbocastana	24	—	—	—	—
Pinnatisecta	24	—	—	—	—
Cardiophylla	24	36	—	—	—
Demissa	24	—	—	60	72
Longipedicellata	—	36	48	—	—
Polyadenia	24	—	—	—	—
(*b*) South America					
Conicibaccata	—	—	48	—	—
Etuberosa	24	—	—	—	—
Commersoniana	24	36	—	—	—
Acaulia	—	—	48	—	—
Cuneoalata	24	—	—	—	—
Tuberosa	24	36	48	60	—

In species evolution the diploids are generally considered to be more primitive, the polyploids being derived from the diploids by chromosome doubling, by hybridization or by a combination of both those methods. Most of the series possess diploid species and these diploid species are far more numerous than any of the others. In some series, indeed, all species are diploid (*Bulbocastana*, *Pinnatisecta*, *Etuberosa*, *Cuneoalata*) whilst in others, a few triploids are also present, formed probably by an unreduced gamete from one plant being fertilized by, or fertilizing, a reduced gamete from another (*Cardiophylla*, *Commersoniana*). A third type is where only tetraploid species are known as in *Conicibaccata*, *Acaulia*, and *Longipedicellata* (the one triploid in this latter series, *S. vallis-mexici*, is possibly a hybrid between this series and some species from *Demissa*). In this case, either the diploid species have not yet been found, or they have already died out, leaving behind the more successful tetraploid derivatives.

In a series such as *Demissa* we have the primitive diploid species but, so far as is known, no triploids or tetraploids. The hexaploid *S. demissum* is well known, but its origin is problematical. Some of the pentaploids are probably inter-series hybrids between *S. demissum* and introduced cultivated tetraploids (vars. of *S. andigenum* are known in Mexico but it is not certain whether they are truly indigenous). The pentaploid *S. semi-demissum* may be formed from *S. demissum* or may on the other hand be a stage in the formation of that species.

With *Tuberosa*, a complete range from diploid to pentaploid is known. Most wild species are diploid, though a few triploids, such as *S. Maglia* and tetraploids such as *S. sucrense*, *S. subandigenum* and *S. leptostigma* are to be met with. These tetraploids are weed species and

are probably very closely related to the cultivated potatoes though we do not know which was derived from which. The one pentaploid in this series is the cultivated *S. curtilobum*. It is assumed to have originated from a cross between *S. andigenum* and *S. Juzepczukii*, the former furnishing a reduced ($n=24$) gamete, the other an unreduced triploid one.

From the information now available to us we can arrive at a very tentative idea of the inter-relationships of the species, based on morphology, crossability studies and disease resistance data. Our knowledge of most of the series is as yet very incomplete, and I have represented their relationships (see Fig. 4) only in a very tentative way by means of dotted lines. The stellate corolla series have been placed in the lower half and the rotate corolla series in the upper half of the figure, assuming that the stellate corolla is a more primitive feature. One cannot attempt to guess at the origin or affinities of the series *Juglandifolia*, *Etuberosa*, *Conicibaccata* and *Polyadenia*, though they all possess a more or less rotate corolla. Neither is

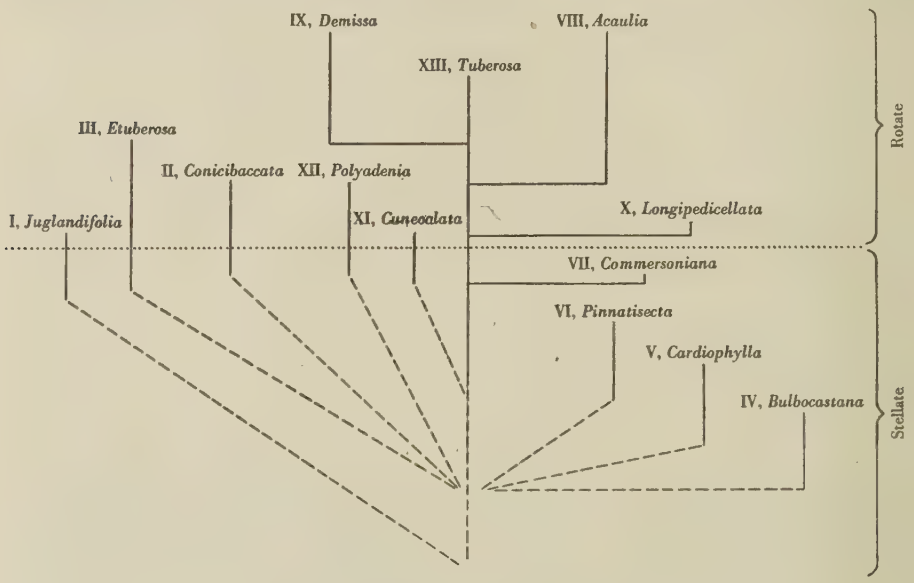

Fig. 4. Possible affinities of potato species groups.

it possible to determine the origins of the series *Pinnatisecta*, *Cardiophylla* or *Bulbocastana* though one can guess at some sort of mutual relationship. Probably more data may be forthcoming shortly when the crossability survey recently started at Cambridge has progressed further. Series *Demissa*, *Acaulia*, *Tuberosa*, *Longipedicellata* and *Commersoniana* will for the most part form inter-series hybrids so that one can assume, I think, that they are more closely related. *Cuneoalata* would seem to be related to the *Tuberosa* stock on morphological grounds but so far has not been hybridized with any other series.

The ultimate object of the potato breeder, in dealing with these wild species, is to make successful hybrids between *Tuberosa* and any species of value in series other than *Tuberosa*. One must obtain the factors for high yield and palatability from the cultivated species in *Tuberosa*, so that sooner or later crosses must be made into this series. The ease with which this can be done I have attempted to illustrate in the figure. Those series that spring off closest to *Tuberosa* are the easiest to cross with it, whilst those that stem off at a greater

distance are relatively more difficult; those that are not joined to the *Tuberosa* stock by a continuous line have so far proved impossible to cross with it. The data for this season (1947) have not yet been worked over, however, and there are indications that crosses may be obtained exceptionally with other series also.

Enough has been said, I think, to provide an outline of the type of framework into which the detailed studies on disease resistance fit. We are still, largely owing to restricted facilities during the war, in the early stages of our studies on the indigenous potatoes, and later investigations will undoubtedly clear up many points that so far remain obscure.

BLIGHT IN RELATION TO POTATO BREEDING

By William Black, *Scottish Plant Breeding Station, Corstorphine, Edinburgh*

The common strain, *A*, of the blight fungus and two new strains, *B* and *C*, were employed in testing potato seedlings (plants but not their tubers) for resistance to the disease. These seedlings were bred from the blight-immune wild species *Solanum demissum* and they were found to consist of five different phenotypes, viz.

(1) Plants immune from *A*, *B* and *C*.
(2) Plants immune from *A* and *B* but susceptible to *C*.
(3) Plants immune from *A* and *C* but susceptible to *B*.
(4) Plants immune from *A* but susceptible to *B* and *C*.
(5) Plants susceptible to *A*, *B* and *C*.

Thus all plants possessing immunity from the *B* or *C* strains were also immune from *A*. Strains *B* and *C* may therefore be regarded as more virulent than *A*, but the difference between *B* and *C*, on the evidence of groups (2) and (3), is qualitative rather than quantitative.

In 1946 a fourth strain *D* was isolated. It appeared on a selection which was immune from the *A* strain but it failed to attack the *B* and *C* filter varieties, i.e. groups (2) and (3). In degree of virulence, the *D* strain appeared to lie either between strains *A* and *B* or between strains *A* and *C*. It seemed probable that, if its virulence could be increased it might be established on either the *B* or the *C* filter plant and repeated inoculations of detached leaves were made for that purpose. Eventually a few conidia were obtained on a leaf of a *C* filter plant and this culture was used for reinoculation of similar leaves. After four passages through these leaves the reaction obtained was similar to that produced by the *C* strain. The *D* strain thus appeared to be a closely related but weak form of *C*, differing from it only in degree of virulence, i.e. quantitatively.

The plasticity of the blight fungus is now widely recognized and many strains of it have been reported. These biotypes, however, apparently do not exist as such in crops of ordinary blight susceptible commercial varieties, since in a survey of blight in Scotland the presence of the *A* strain only was recorded.

Inheritance of resistance. In so far as the *A*, *B* and *C* strains are concerned, blight resistance is a heritable character, behaving in dominant fashion, and it appears to be controlled by major genes as follows:

Ra confers immunity from strain *A*.
Rb confers immunity from strains *A* and *B*.
Rc confers immunity from strains *A* and *C*.
Rbc confers immunity from strains *A*, *B* and *C*.

In addition, minor genes appear to be present which determine the degree of susceptibility in susceptible varieties and act as modifiers in resistants.

Segregation of immunes and susceptibles in progenies invariably show an excess of recessives compared with standard Mendelian ratios.

Table 1

Mendelian ratios	Observed ratios	
	Strain *A*	Strain *B*
1 : 1	0·85 : 1	0·88 : 1
3 : 1	2·36 : 1	2·27 : 1
7 : 1	6·00 : 1	6·18 : 1
15 : 1	10·14 : 1	11·50 : 1

These unbalanced ratios, shown in Table 1, may be due in part to chromosome homologies leading to multivalent formation and double reduction, but, in view of the incompatibility factors present in the original wild species, they are more likely to be caused by differential compatibility of gametes. Blight immunity is a 'wild' character and is therefore expected to be associated in some degree with any incompatibility factors inherited from *S. demissum*.

Comparison of strains A *and* B. The presence of *Ra* or *Rb* genes in parent varieties was ascertained by submitting progenies to a double test in which the plants were inoculated with strain *A* to eliminate the *A* susceptibles, and the survivors with strain *B* to eliminate the types possessing immunity from *A* and susceptibility to *B*. Results of such tests are shown in Table 2. In the first six cases no plants were killed by the *B* strain, therefore the immune parents had *Rb* but not *Ra* in their constitution. In the remaining progenies both the *Ra* and *Rb* genes were present.

Table 2

Parentage	No. of seedlings				Ratio		Dominant genes
	Tested	Killed by *A*	Killed by *B*	Survived	*A* test	*B* test	
S × 1257*a* (7)	201	97	0	104	1·07 : 1	1·07 : 1	*S* × *Rb*
S × 1335*a* (5)	221	105	0	116	1·10 : 1	1·10 : 1	*S* × *Rb*
1256*a* (23) × *S*	94	50	0	44	0·88 : 1	0·88 : 1	*Rb* × *S*
S × 1307*a* (23)	126	18	0	108	6·00 : 1	6·00 : 1	*S* × Rb_1 Rb_2 Rb_3
1256*a* (23) × 1307*a* (23)	150	12	0	138	11·50 : 1	11·50 : 1	*Rb* × Rb_1 Rb_2 Rb_3
1253*a* (15) × 1307*a* (23)	102	6	0	96	16·00 : 1	16·00 : 1	Rb_1 Rb_2 × Rb_1 Rb_2 Rb_3
S × 1306*a* (2)	330	99	59	172	2·33 : 1	1·09 : 1	*S* × *Ra Rb*
882 (5) × *S*	369	98	92	179	2·77 : 1	0·94 : 1	*Ra Rb* × *S*
882 (5) × 834*c* (29)*	215	40	73	102	4·37 : 1	0·90 : 1	*Ra Rb* × *Ra*

S = susceptible variety. * = 834*c* (29) was immune from *A* and susceptible to *B*.

Comparison of strains A, B *and* C. The ratios obtained when similar progenies were tested with the *A*, *B* and *C* strains, indicated the identity of the genes involved. Seedling 997*a* (51) (Table 3) which was immune from all three strains gave, on crossing with a susceptible variety, similar ratios in each case. The gene concerned was therefore *Rbc*.

Seedling W800 (2) which was immune from *A* and *C* but susceptible to *B*, gave comparable results with the *A* and *C* strains. The dominant gene was therefore *Rc*.

In the case of 885 (2) the ratios obtained in both the *A* and the *C* tests were approximately 3 : 1, but when tested with strain *B* approximately equal proportions of immunes and susceptibles resulted. Seedling 885 (2), which is immune from all three strains, is therefore represented by *Rc Rbc*.

A change in virulence? Several seedlings related to series 1104 survived the routine test with the *B* strain in 1946 but in 1947 they gave a susceptible reaction. This unexpected result

was not satisfactorily explained until some of the 1946 progeny tests were repeated in 1947. The details of two crosses tested in 1946 and repeated in 1947 are shown in Table 4.

The constitution of 882 (5) was established in other tests and it behaved normally in both seasons. The ratios obtained in progenies bred from 1104*c* (2) suggest that this variety had lost an *Rb* gene, but since some of the seed used in 1947 was the same stock as that used in 1946, this explanation was not acceptable.

A further progeny derived from *S* × 1104*c* (2) was raised in 1947 and subjected to a double test with strains *A* and *B*. The results are shown in Table 5.

Table 3

Parentage	Strains	No. of seedlings: Immune	No. of seedlings: Susceptible	Ratio	Dominant genes
S × 997*a* (51)	*A*	1399	1569	0·89 : 1	*S* × *Rbc*
	B	187	212	0·88 : 1	
	C	100	107	0·93 : 1	
S × W800 (2)	*A*	902	1245	0·72 : 1	*S* × *Rc*
	B	0	350	0·00 : ∞	
	C	75	94	0·80 : 1	
S × 885 (2)	*A*	216	75	2·88 : 1	*S* × *Rc Rbc*
	B	125	124	1·00 : 1	
	C	84	26	3·23 : 1	

Table 4

Parentage	Year of test	No. of seedlings: Immune	No. of seedlings: Susceptible	Ratio	Dominant genes
S × 1104*c* (2)	1946	653	311	2·10 : 1	*S* × Rb_1 Rb_2
	1947	715	767	0·93 : 1	*S* × *Rb*?
882 (5) × 1104*c* (2)	1946	469	77	6·09 : 1	*Ra Rb* × Rb_1 Rb_2
	1947	194	67	2·89 : 1	*Ra Rb* × *Rb*?

Table 5

Parentage	No. of seedlings: Tested	Killed by *A*	Killed by *B*	Survived	Ratio: Under *A* test	Under *B* test	Dominant genes
S × 1104*c* (2)	276	94	56	126	2·0 : 1	0·84 : 1	*S* × *Ra Rb*

Thus the double test in 1947 showed that 1104*c* (2) carried the two genes *Ra Rb*. Since this plant was credited with Rb_1 Rb_2 in 1946 it would seem that one of these genes had provided only minimum protection against the *B* strain in 1946 and that the *B* strain had increased in virulence in 1947 to an extent sufficient to overcome the normal resistance conferred by that gene. In view of the acknowledged plasticity of the fungus and the evidence of related seedlings this explanation appears to meet the circumstances.

Such evidence of an increase in virulence of strain *B* was confined to the few progenies related to series 1104, presumably on account of the comparatively low level of protection provided by that particular gene. The resistance conferred by all other *Rb* genes in the experiments must necessarily be of a higher order.

It may be concluded, first, that qualitatively different strains of blight may develop, as exemplified by *B* and *C*; and secondly, that quantitative differences in virulence may be exhibited in the development of these qualitatively different lines, as indicated by the plasticity of *B* and *D*. The ultimate number of recognizable strains must presumably be large and would depend upon the range of test plants available for differentiation of the fungus.

SOME RECENT RESULTS OF POTATO DRY ROT RESEARCH

By A. E. W. Boyd, *Plant Pathology Laboratory, Department of Agriculture for Scotland, East Craigs, Corstorphine, Edinburgh*

Dry rot has always caused substantial losses in seed potato tubers, particularly of early varieties. In recent years the disease has gained widespread prominence because of very severe losses in the variety Doon Star. This is the principal reason for the enormous decrease in acreage of this variety inspected in Scotland in the past few years. In 1942, with just over 12,000 acres, it was second only to Majestic in Scotland. In 1946, this figure had dropped to 1960—a decrease of 83 %—the variety being 12th on the list. The Majestic acreage continued to increase for 2 years after Doon Star had reached its peak, and since then has fallen by only 17 %.

A co-operative investigation on potato dry rot was commenced in 1941 by the Agricultural Research Council and the Department of Agriculture for Scotland, and some of the results of this work, particularly of experiments to control the disease and to obtain information on the time and methods of infection, are presented in this paper. Fuller details of this work will be published in due course.

Time and method of dry rot infection

Laboratory experiments

It has long been believed that *Fusarium caeruleum*, the principal organism responsible for the disease in this country, is essentially a wound parasite. This was illustrated by an experiment where samples of Arran Pilot and Doon Star tubers were individually subjected to controlled mechanical wounding and bruising and inoculated immediately with a spore suspension of *F. caeruleum* on the site of the wound or bruise. The skin of the tubers was never broken by the slight bruise but in almost all cases a fairly deep wound was caused by the severe type, while the intermediate bruise broke the skin in most cases but the wound was not deep. The percentage of dry rot developing on the slight, intermediate and severe bruises was respectively 0, 13 and 55 in the variety Doon Star, and 1, 21 and 59 in the variety Arran Pilot. It appears, therefore, that unless the skin is broken a bruise alone does not permit the fungus to penetrate the tuber under normal conditions.

Several workers have concluded that little if any infection occurs in ordinary field crops prior to lifting, but have indicated that infection of unbruised tubers can apparently occur. In one experiment it was observed that although there was extremely little spread from a diseased tuber acting as an infector within an individual box, occasionally spread by contact did apparently occur. Whether a wound is absolutely essential therefore is doubtful and it may be that a local poisoning action on the part of the rotting tuber would permit the entrance of the fungus into an adjacent healthy tuber. Such instances, however, have been very uncommon.

That the fungus does not apparently penetrate newly formed callus tissue was shown in an experiment where a standard spore suspension was applied to standard wounds at intervals up to 3 months. The results indicated that even a severe wound is no longer susceptible to dry rot attack between 2 and 8 days after being formed. Obviously the time for callus formation will vary with different environmental conditions, but once the callus layer has been completed infection is apparently prevented.

The only other avenue which has been found to lead to dry rot infection to any extent is through lesions of powdery scab (*Spongospora subterranea*). Counts made in one experiment showed that in one stock of Arran Pilot, 77 % of the dry rot lesions originated from, or were associated with, powdery scab pustules. *Phoma foveata* infection has also been observed to originate from powdery scab pustules in this country.

An investigation of other sources of infection showed that contaminated boxes or contaminated stores played no material part in the development of dry rot if the tubers were undamaged.

Field experiments

These experiments were conducted over a period of several years, and the results using a number of susceptible varieties confirm that the only method of penetration of *F. caeruleum* of any practical significance is through wounds and abrasions. They also indicate that damage sustained on the riddle is by far the most important factor. When this is appreciated, the origin of the belief that dry rot did not develop to any extent in a clamp until the clamp was opened can readily be understood.

One experiment of this type showed (1) that digger and associated lifting injury is often only a relatively minor factor in the penetration of dry rot, (2) that riddling damage is of major importance, (3) that there is a possibility of considerably reducing dry rot losses by a different system of dressing, (4) that rough handling of the bags can in some cases double the number of diseased tubers and (5) that it is possible to vary the amount of dry rot in one stock of tubers from 3 to 42% merely by altering the handling conditions. The amount of dry rot developing after hand dressing was 4%, and after machine riddling 16%, while tubers twice riddled developed 24% dry rot.

In general, the effect of transport appears to be more in the nature of a secondary effect upon potatoes which have been riddled and is relatively insignificant on hand-dressed tubers.

The environment of the tubers immediately following the period of mechanical damage was found to be of practical importance. Bagging or clamping instead of boxing subsequent to riddling tends to increase the amount of dry rot, presumably because the higher humidity encourages the development of the disease. It was shown by experiment that if tubers were wetted prior to riddling the amount of dry rot which developed in bags increased from 16 to 23%. In this connexion it is interesting to observe that probably the only time the Scottish grower becomes aware of dry rot, although not recognizing it as such, is when he reclamps the ware of susceptible varieties after removing the seed and despatching it to England.

It was found in several experiments that the locality of storage either in England or Scotland had no effect on the development of dry rot.

Chemical control

Dips

A very satisfactory level of control was achieved by dipping tubers in organo-mercury solutions immediately after the tubers had been lifted and dressed, and storing them subsequently in boxes. Formalin appeared to give a less efficient control. Results were similar whether tubers were despatched to England in December or in March. A considerable reduction in the amount of disease was obtained by dipping tubers on riddling prior to despatch to England, the tubers having been clamped on lifting. The level of control, however, was not on the average as high as that obtained by the previous method.

It is interesting to examine these results in the light of the evidence presented previously on the importance of riddle damage in relation to dry rot development. The dipping on riddling presumably did not control infection on lifting injuries, but obviously these were not of a very high order and it was successful in preventing a further increase which would have developed from riddle wounds. This was further borne out by the fact that treatment immediately on receipt in England rarely provided any control whatsoever. Moreover, dipping of tubers on lifting, storing them in clamp and then riddling in December provided a variable but normally poor degree of control.

The separation of the relatively small amount of infected tubers which occurred in the dipped series into those where the lesion apparently arose from a gross wound and those where no such connexion was obvious was carried out in a number of experiments and showed that more than 50% of the 'uncontrollable' dry rot amongst dipped tubers is associated with gross wounds, which suggests that the fluid fails to penetrate a large proportion of cracks or deep wounds. Observational evidence supports this view.

In spite of the control achieved by dipping at the correct time the difficulties involved in the dipping process are such that it is thought unlikely ever to provide the answer to the dry rot problem.

Dusts

The wide adoption of any method of chemical control of dry rot will depend on (1) effectiveness, (2) ease of application, and (3) small capital outlay. Only a dry treatment would appear likely to fulfil all these conditions. Preliminary small-scale tests showed that thymol provided an efficient control and was also in other ways very satisfactory.

Large-scale trials were commenced using thymol first of all at the rate of 12 oz. made up to 10 lb. with kaolin per ton of potatoes on 'as grown' tubers prior to clamping. This does not interfere with Scottish farming practice but necessitates the use of a compound harmless both to human beings and to stock on account of the incidental treatment of ware. From several preliminary tests thymol appeared to be satisfactory in this respect. However the degree of control effected using this method was in general rather variable. In further experiments tubers were clamped on lifting and treated immediately after riddling, prior to despatch to England. A variable degree of control was achieved in this way but again it was not entirely satisfactory.

These two methods were then combined by application of the compound both on lifting as the tubers were being collected into the carts in the field, and again immediately the tubers had passed over the riddle and were dropping into the sacks. The compound can thus act at the times of maximum damage to the tubers. The results obtained, using several varieties, Arran Pilot, Catriona and Doon Star, indicated a highly satisfactory control of the disease, in one case even with a lower thymol rate (6 oz. in 10 lb. kaolin) the decrease in dry rot development was from 16 to 0·3 %. It is possible that the effect of the second application may also be maintained in the bags during the time the tubers are subject to transport damage.

Various other carriers were also tested, for example, fine and coarse granulated peat, peat dust, kieselguhr, gypsum and wood meal but none gave such satisfaction as kaolin.

Unfortunately, thymol occasionally had a marked phytocidal effect on the tubers. In severe cases the tuber was disfigured and rendered unsaleable, but although a few blind tubers were observed, trials with severely damaged tubers showed no decrease in yield. In some very severe cases, however, the whole tuber became soft and eventually shrivelled completely. It was found that early lifted or immature tubers of the varieties tested (Arran Pilot, Doon Star, Sharpe's Express, Catriona and Ninetyfold) were much more susceptible to thymol damage than mature tubers. None of the late-lifted samples showed any marked injury. Thymol was also shown to act upon wounds and abrasions already present on the tubers, the injury on riddled tubers being noticeably higher than on hand-dressed tubers. This damage was mainly in the 'slight' category. The damage in all cases was rendered much more severe when tubers were wetted prior to treatment.

Variations in susceptibility to thymol damage were found between different varieties and of those tested Ninetyfold and Arran Pilot were most liable to injury while Doon Star was least susceptible. This order is similar to that of the susceptibility of these varieties to any other form of damage.

An attempt was made to use reduced rates of thymol so as to avoid damage completely, but even with 1 oz. thymol per ton some slight effect was noted.

In several small-scale laboratory tests it was found that thymol vapour could inhibit almost completely the growth of even large colonies of *Fusarium caeruleum*. In some cases after 8–14 days the colonies were killed. It is believed, therefore, that if the vapour is present in sufficient concentration a certain amount of surface sterilization can result but it is not known, with certainty, whether in practice such a concentration is effectively maintained so that the substance can act as fungicide, or whether it acts merely as a fungistat for a period long enough for callus formation to occur on wounded tissues.

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1942 CLAYSON, D. H. F., M.Sc., F.R.I.C., A.R.C.S., D.I.C., The Laboratories, J. Lyons and Co., Ltd., Cadby Hall, London, W. 14.
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1920 FOX-WILSON, G., N.D.H., F.L.S., R.H.S. Laboratories, Wisley, Ripley, Woking, Surrey. (General and Zoological Secretary 1935–6; Zoological Secretary 1933–4, 1937–8; Council 1927–9.)

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1913 FRYER, Sir JOHN, K.B.E., O.B.E., M.A., Agricultural Research Council, 6*a* Dean's Yard, Westminster, London, S.W. 1. (President 1926–7; Treasurer 1914–19; Council 1921, 1924–5.)

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1920 GIMINGHAM, C. T., O.B.E., B.Sc., F.R.I.C., Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (President 1938–40; Vice-President 1937; Zoological Editor 1934–44; Council 1929–32.)

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1939 GREENHILL, A. W., Ph.D., 25 Meadow Walk, Harpenden, Herts.

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1941 HERFORD, G. V. B., O.B.E., B.A., M.Sc., Director, Pest Infestation Laboratory, Slough, Bucks. (Council 1944–6; Secretary 1947– .)
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1930 MARTIN, H., D.Sc., A.R.C.S., F.R.I.C., Research Station, Long Ashton, nr. Bristol. (President 1941–2; Vice-President 1938; Council 1936–8.)
1940 MARTIN, J. T., D.Sc., F.R.I.C., Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (Council 1945–7.)
1922 MASON, E. W., M.A., M.Sc., F.L.S., Commonwealth Mycological Institute, Kew, Surrey. (Vice-President 1943–4; Council 1943–5.)
1927 MASSEE, A. M., D.Sc., East Malling Research Station, nr. Maidstone, Kent. (Council 1932–4.)
1946 MATTHEWS, R. E. F., M.Sc., Plant Virus Research Unit, Molteno Institute, Cambridge.
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